ADVANCES IN INTERNAL MEDICINE

ADVANCES IN INTERNAL MEDICINE

VOLUMES I and II (out of print)

VOLUME III

VOLUME IV

HEPATIC TESTS, *by Hans Popper and Fenton Schaffner*

THE VASCULAR PHYSIOLOGY OF HYPERTENSION, *by G. W. Pickering*

VOLUME V

DISEASES OF THE PREGNANT WOMAN AFFECTING THE OFFSPRING, *by Murray H. Bass*

CATHETERIZATION OF THE HEART, *by Richard J. Bing*

PORTAL HYPERTENSION AND ITS TREATMENT, *by Arthur H. Blakemore*

THE ANEMIA OF INFECTION: XVII. A REVIEW, *by G. E. Cartwright and M M. Wintrobe*

GOUT, A DERANGEMENT OF PURINE METABOLISM, *by Alexander B. Gutman and T F. Yu*

CLINICAL ASPECTS OF GANGLIONIC AND ADRENERGIC BLOCKING AGENTS, *by Richard H Lyons and V Logan Loce*

ASPECTS OF THE INFLUENZA PROBLEM, *by J Mulder*

EXPERIENCES WITH ADRENOCORTICOTROPIC HORMONE (ACTH) AND CORTISONE, *by Charles Ragan*

ABNORMAL PROTEINS IN MYELOMA, *by J. Waldenstrom*

ADVANCES *in* INTERNAL MEDICINE

EDITORS

WILLIAM DOCK, M.D.
Long Island College of Medicine, Brooklyn

I. SNAPPER, M.D.
Beth-El Hospital, Brooklyn

VOLUME VI · 1954

THE YEAR BOOK PUBLISHERS · INC.

For Great Britain and Northern Ireland
Interscience Publishers, Inc
88-90 Chancery Lane, London, W C 2

PRINTED IN U.S.A.

Table of Contents

Uropepsin*

DONALD C. BALFOUR, JR.

University of Southern California School of Medicine, and the Los Angeles County Hospital, Los Angeles

IN THE PAST few years there has been an increasing interest in the quantitative measurement of the peptic activity of acidified urine. Knowledge of this enzymic activity in the urine contributes to a better understanding of gastric secretory function in health and disease and provides a method of measuring the response to stress situations and the reactions of the body An attempt will be made here to summarize the working knowledge of this subject for the clinician.

The substance has been referred to in the literature as uropepsin (9), uropepsinogen (24), urinary pepsinogen (21), urinary renninogen (34), or just peptic-like activity of the urine at acid reactions. Uropepsin was the name first given to this enzymic activity in the urine and this name is used here One reason for the confusion in nomenclature is that, until activation by strong acidification, only the precursor of this enzyme exists in urine. When activated, the enzyme is practically identical with the pepsin of the stomach, the precursor of which is pepsinogen A further source of confusion is the two tests used to measure this enzymic activity One test measures the proteolytic activity of the enzyme, the other, its ability to coagulate milk casein or its rennin-like activity Rennin itself is not thought to exist in the adult (22) Measurement of the proteolytic action or the rennin action gives comparable results, and proteolytic enzymes other than uropepsin do not significantly change results as assayed with the methods mentioned below. The name uropepsin, however, suggests that pepsin is normally

* Original work presented from this institution was assisted in part by the California Division of the American Cancer Society and in part by the Deborah Fox Fund.

present in the urine when, in fact, only its precursor, pepsinogen, is present.

PHYSIOLOGIC BACKGROUND

Excellent review articles about uropepsin are available (1, 9, 21); here only the particular works identifying the physiologic principles will be mentioned. After its discovery in 1861 (8), uropepsin was shown in 1904 to be dependent on the stomach when Frouin (14) found that the enzyme disappeared from the dog's urine after total gastrectomy. It has since been repeatedly proved that this enzyme arises solely from the peptic cells of the stomach.

Frouin also found that a dog with a total external pouch of the stomach had normal levels of uropepsin even though all the gastric excretions were lost from the pouch outside the body. This study demonstrated that uropepsin originates from the peptic cells of the stomach as an endocrine secretion of the stomach.

To elucidate the source and transfer of this enzymic activity from the stomach to the urine, Mirsky *et al.* (29) gave pepsin by mouth and injected pepsin into the blood stream of dogs; no change in uropepsin levels in the urine resulted. However, when crude pepsinogen was injected intravenously there was a rapid rise and fall in urinary uropepsin Mirsky *et al.* (27, 30) also assayed the plasma of normal subjects, of patients with pernicious anemia and of patients with duodenal ulcers and found the variations in amounts to be comparable to the amounts of uropepsin present in these conditions. Both Bucher (9) and Mirsky *et al.* (29) have demonstrated that urinary and plasma pepsinogen are identical with crystalline pepsinogen obtained from the stomach These studies more completely establish the fact that the only source of uropepsin in the urine is the endocrine secretion of pepsinogen from the stomach into the body fluids and blood stream.

The demonstration that pepsinogen injected intravenously appears as uropepsin within 24 hours and that uropepsin disappears from the urine within 24 to 48 hours after total gastrectomy (5) proves that there is a relatively free overflow through the kidneys into the urine Furthermore, no retention of uropepsin has been noted in patients with considerable kidney damage (3), nor is uropepsin influenced by variations in volume, urinary pH, or specific gravity of the normal urine (9, 29)

While it is possible, in theory, that some inhibitors of pepsinogen may be present in urine, Mirsky *et al.* (29) showed this not to be the

case. If inhibitors were present, the enzymic activity would decrease over a period of time; but several workers have shown that substantially the same activity remains for up to 4 days at room temperatures or for 2 weeks at 4 C (9, 16, 29) even in the absence of preservatives (16). In the absence of destruction of the enzyme in the urine, or of any known catabolism while the enzyme is in the body, and with free over-

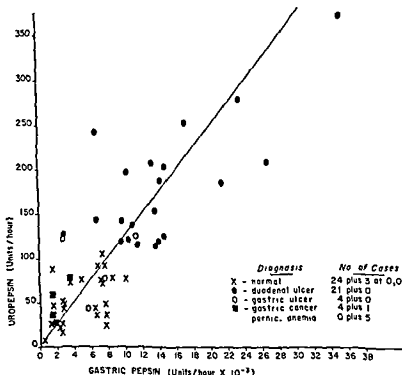


FIG 1 —Scatter diagram showing relation of gastric pepsin secretion to urinary pepsinogen excretion in 62 human subjects Nine points fell at the origin (0, 0) and are therefore not shown (24)

flow through the kidney, the finding of low amounts or none at all in the urine can only mean that the enzyme is not being produced by the stomach.

Janowitz and Hollander (24) correlated uropepsin values with the amount of pepsin excreted into the stomach Careful measurement of the amount of pepsin in the stomach and uropepsin in the urine over the same period revealed that approximately 1 per cent of the pepsinogen was secreted into the body fluids and the urine, while 99 per

present in the urine when, in fact, only its precursor, pepsinogen, is present.

PHYSIOLOGIC BACKGROUND

Excellent review articles about uropepsin are available (1, 9, 21); here only the particular works identifying the physiologic principles will be mentioned. After its discovery in 1861 (8), uropepsin was shown in 1904 to be dependent on the stomach when Frouin (14) found that the enzyme disappeared from the dog's urine after total gastrectomy. It has since been repeatedly proved that this enzyme arises solely from the peptic cells of the stomach.

Frouin also found that a dog with a total external pouch of the stomach had normal levels of uropepsin even though all the gastric excretions were lost from the pouch outside the body. This study demonstrated that uropepsin originates from the peptic cells of the stomach as an endocrine secretion of the stomach.

To elucidate the source and transfer of this enzymic activity from the stomach to the urine, Mirsky *et al* (29) gave pepsin by mouth and injected pepsin into the blood stream of dogs, no change in uropepsin levels in the urine resulted. However, when crude pepsinogen was injected intravenously there was a rapid rise and fall in urinary uropepsin. Mirsky *et al.* (27, 30) also assayed the plasma of normal subjects, of patients with pernicious anemia and of patients with duodenal ulcers and found the variations in amounts to be comparable to the amounts of uropepsin present in these conditions. Both Bucher (9) and Mirsky *et al.* (29) have demonstrated that urinary and plasma pepsinogen are identical with crystalline pepsinogen obtained from the stomach. These studies more completely establish the fact that the only source of uropepsin in the urine is the endocrine secretion of pepsinogen from the stomach into the body fluids and blood stream

The demonstration that pepsinogen injected intravenously appears as uropepsin within 24 hours and that uropepsin disappears from the urine within 24 to 48 hours after total gastrectomy (5) proves that there is a relatively free overflow through the kidneys into the urine. Furthermore, no retention of uropepsin has been noted in patients with considerable kidney damage (3), nor is uropepsin influenced by variations in volume, urinary pH, or specific gravity of the normal urine (9, 29).

While it is possible, in theory, that some inhibitors of pepsinogen *may be present in urine, Mirsky et al (29) showed this not to be the*

case. If inhibitors were present, the enzymic activity would decrease over a period of time, but several workers have shown that substantially the same activity remains for up to 4 days at room temperatures or for 2 weeks at 4 C (9, 16, 29) even in the absence of preservatives (16). In the absence of destruction of the enzyme in the urine, or of any known catabolism while the enzyme is in the body, and with free over-

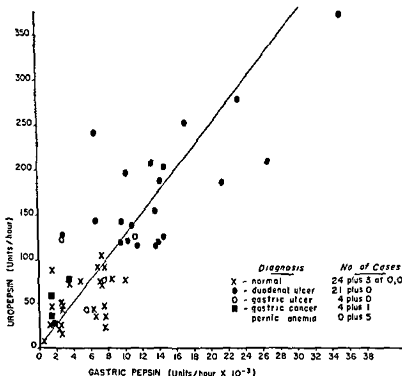


FIG 1 —Scatter diagram showing relation of gastric pepsin secretion to urinary pepsinogen excretion in 62 human subjects Nine points fell at the origin (0, 0) and are therefore not shown (24)

flow through the kidney, the finding of low amounts or none at all in the urine can only mean that the enzyme is not being produced by the stomach

Janowitz and Hollander (24) correlated uropepsin values with the amount of pepsin excreted into the stomach Careful measurement of the amount of pepsin in the stomach and uropepsin in the urine over the same period revealed that approximately 1 per cent of the pepsinogen was secreted into the body fluids and the urine, while 99 per

cent was excreted directly into the stomach. Increased amounts of pepsin in the stomach were associated with high uropepsin values and vice versa (Fig. 1).

This classic experiment supports and demonstrates Gottlieb's (17)

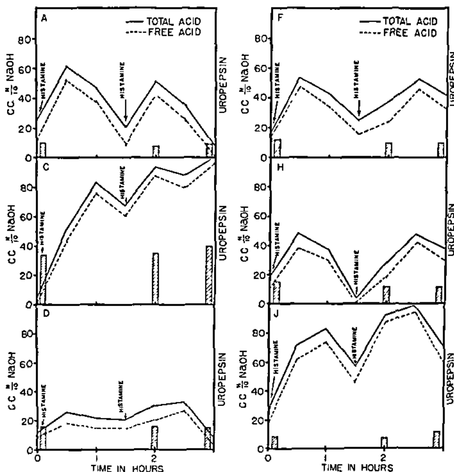


FIG. 2 —Simultaneous uropepsin determinations and histamine gastric analysis. No rise in uropepsin occurred subsequently. The same scale represents both acid and uropepsin values

theory. The theory had been generally accepted, but was only proved by the work of Janowitz and Hollander. Their investigation is the most convincing correlation that has been given in the literature.

Usually, in the presence of high peptic activity uropepsin values are elevated along with hyperacidity in the stomach. But it is well known that histamine can raise the acid secretion in the stomach

without an increase in pepsin or uropepsin (10). Simultaneous determinations in normal individuals of uropepsin levels and of gastric acid excreted after histamine injection reveal no change in uropepsin during the hyperacidity response (Fig. 2). On the other hand, individuals with normally high uropepsin levels show sustained elevation of acid by the histamine test, while most of those with histamine achylia excrete minimal amounts of uropepsin It is now evident that the peptic activity or total peptic function of the stomach can be estimated approximately by measuring the quantity of uropepsin in the urine. It is this part of stomach function which will be considered below

ASSAY METHODS

In her comprehensive review of the literature Bucher (9) discussed the development of enzyme chemistry in regard to uropepsin. Interest in uropepsin as a measurement of gastric function was markedly delayed because of the complexity of the chemical procedures and inability to standardize results.

The most widely used method of assay for uropepsin since the introduction of the Folin-Ciocalteu (13) reagent in 1927 is that of Anson and Mirsky (2). A carboxyhemoglobin substrate is digested for a given length of time by a specimen of urine containing the enzyme and by one with the enzyme deactivated. The amount of enzyme can be measured by the difference between the 2 specimens in amount of digestion or release of tyrosine. Since then, other workers have used this technic with various modifications convenient to their laboratories. The measurement is done at pH 1 5 to 1.8.

This method is the most exact biochemical technic at our disposal, but for practical use the obtaining of the substrate and the laboratory procedure are both difficult There has been considerable variation in the units used to express results among the laboratories that have used this procedure and mastered a technic which gives them consistent results The results have been expressed in milligrams of tyrosine excreted per hour, per 24 hours, per cubic centimeter, or per period of incubation It is therefore difficult to compare results from one laboratory with those of others. This exact biochemical technic, however, has been and will continue to be the real basis for the determination of uropepsin in investigative work

West *et al* (36) have reported a much simpler laboratory method for measuring uropepsin by determining the rennin activity of the urine. For this method, easily obtained materials can be used.

Some (26, 32) have questioned whether the rennin property of the urinary enzymic activity actually measures uropepsin the way the Mirsky method does. The results are so similar, however, and the simplicity of the test is so important for general use, that until better assay procedures are found this rennin method may be considered an adequate measure of gastric function. We have found the milk coagulation test to give the same comparative readings as the Mirsky technic on the same urine specimens, and our determinations have therefore been done with this easier technic. The test is based on the number of seconds it takes an activated urine sample to cause aggregation of casein particles in a milk solution No blank run is necessary because a nomogram is available (36) from which a direct reading in number of seconds is made and the result can be immediately calculated. Unit readings of 10 to 40 units per hour are obtained in a normal person by this method with high levels going up to 300 to 400 units per hour. The rennin-like action of uropepsin is determined at pH 4 9 after activation at pH 1.5

Goodman, Sandoval, and Halsted (16) have used different sources of homogenized milk and a standard casein preparation and found the results on the same urine specimen to be generally similar with different brands of milk and invariably similar with the standard casein preparation Sachar and Sicher (32) believe that for general use homogenized milk is as good as the casein preparation The materials for obtaining comparative and quite accurate results are therefore available to any laboratory. Much more can now be learned in regard to uropepsin excretion, for this simpler method makes possible more general clinical study of the enzyme.

It is the rate of excretion of uropepsin that is measured (9, 17), and the unit of measurement is units per hour. This rate of excretion is relatively constant throughout the day. However, we have collected measured 24 hour urine specimens as often as possible and used an aliquot of this specimen for measurement The more convenient "timed and measured" morning collection is a close estimate of the constant activity in the stomach but may be higher in certain individuals than is found by comparison with the 24 hour value (36)

UROPEPSIN EXCRETION

In Normal Individuals

Age —Our observations, like those of Farnsworth *et al* (12), reveal that the rate of uropepsin excretion drops in the older age groups.

Hirschowitz (21) did not find a statistical difference in his results, but his graphs show a scatter which might be interpreted as a tendency toward the same decrease. Such a decrease parallels the accepted decrease of gastric acid in the aged.

Sex.—In most instances, no significant difference has been found between male and female controls. Two studies noted a somewhat lower output of uropepsin in women (11, 26).

Diet.—Bucher's (9) study, of women only, showed that a high protein diet raises the uropepsin level in the urine by the second or third

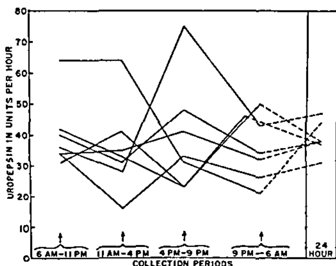


FIG 3.—Diurnal variations in uropepsin output of a normal human subject on seven consecutive days (16)

day A nonprotein diet will decrease the excretion in the same period of time. In our experience, the uropepsin values always fall after weight reduction programs or starvation Ordinary variations in diet do not significantly affect uropepsin levels nor is there any uropepsin increase in response to a meal

Diurnal variations.—Figure 3 shows the changes found by Goodman *et al* (16) throughout the day in the normal subject Other observations reveal similar changes. This variation is unpredictable, but we have suspected that the low levels were in some way related to fatigue The variation in normal subjects from the highest to the lowest value during a day has never been over 50 per cent, in the absence of obvious unusual stress Because there is frequently some variation, most workers use 24 hour measured urine specimens whenever possible

Menstruation.—A slight rise is often noted in women during the premenstrual and menstrual periods.

Pregnancy.—We have noted a slight increase during the entire pregnancy in several normal women, with a decrease to their normal levels after delivery.

IN STRESS REACTIONS

It is well known clinically that the gastric secretions and gastric motility can be stimulated by emotional reactions. Studies of the gen-

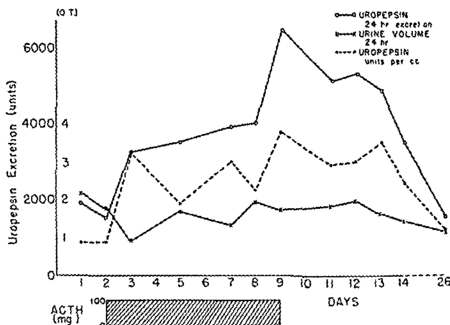


FIG 4—Effect of ACTH on uropepsin excretion in a normal human subject (33)

eral adaptation syndrome have also shown that there are physical or emotional bodily reactions to stress effective primarily through the hormonal axis of the pituitary and adrenal glands Gray *et al* (18) investigated the influence of hormonal factors on the stomach and found low uropepsin excretion in Addison's disease and high uropepsin excretions with pituitary, adrenal, or hypothalamic hyperactivity in the presence or absence of the vagus nerve Spiro *et al.* (33) found, by measuring uropepsin excretion, that the peptic activity of the stomach

is increased when ACTH or cortisone is administered in excessive amounts (Fig 4). Much of the work and interest in the measurement of uropepsin at present are directed toward analyzing the stress reaction pattern of the individual patient.

Is uropepsin increased in response to purely psychic stimuli? There is some evidence to suggest that this may be true. As Gray *et al.* and others have shown, the response to cortisone of individuals with a normal or high uropepsin output is a gradual rise of uropepsin after 24 to 48 hours up to very high levels. Repeated uropepsin determinations two or three times daily on patients waiting for surgery or under observation in the hospital have disclosed that an immediate, marked in-

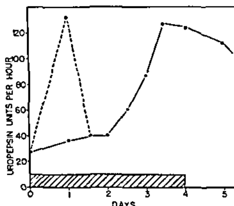


FIG 5—Uropepsin excretion following psychic trauma (dashes) compared with the rise after cortisone administration (solid line). Traumatic episode at zero; cortisone administration, cross-hatched block

crease in uropepsin excretion, returning to normal within 12 hours, can occur following an emotional upset, such as the patient's being told that an operation must be performed or after an argument with relatives (3, 4, 19, 20) Figure 5 illustrates this distinction between the uropepsin response to an emotional stress and the response to adrenal stimulation.

Garst and Hilliard (15), in a prolonged study of sex psychopaths, measured the uropepsin output and 17-ketosteroid excretion before, during, and after stressful situations Depending on the individual's pattern of response to stress (which was unpredictable), the uropepsin excretion varied from high to low values. High and low uropepsin values were found in patients having low 17-ketosteroid excre-

tion. However, no low uropepsin values were found in patients with a high 17-ketosteroid excretion. These observations strongly suggest that emotional distress alone can elevate uropepsin values without increasing adrenal activity, but that when the stress situation also increases adrenal function the uropepsin levels cannot be low.

Mirsky *et al.* (28) determined the uropepsin excretion of patients under psychiatric treatment, and noted the type of emotional stress which is associated with high or low uropepsin excretion in the same individual These results indicate that the emotional problems which have been related to relapses in ulcer patients increase the excretion of uropepsin. Fear and resentment usually are associated with high levels of uropepsin just as these emotions have been related to aggravation of an ulcer Jacobs *et al* (23), measuring the uropepsin excretion in patients with psychoses, found that the levels decreased when the patient began to lose affect or contact with reality As the patient recovered with insulin shock therapy, there was a gradual rise in the uropepsin level.

In Peptic Ulcer and Gastric Cancer

In the differential diagnosis of peptic ulcer and gastric cancer, measurement of uropepsin excretion seems to have about the same value as the determination of gastric acidity. Some of the changes in the functional state of the stomach, as related to the stress situations mentioned above and in Cushing's or Curling's ulcer, are more apparent by this test Furthermore, in certain instances, as during upper gastrointestinal hemorrhage, the uropepsin can be measured when it would be impossible to determine the gastric acids. This has been found to be of value by Eastcott *et al.* (11), although in 2 patients (1 male, 1 female) we have noted that the stress of a bleeding episode caused a marked rise in uropepsin excretion over the individual's normal rate of excretion. The uropepsin levels are about double the normal levels, on the average, in all patients having peptic ulcer (25). Hirschowitz (21), however, reported that the uropepsin levels rise only after the peptic ulcer has been active for 5 years He also reported that the response in benign peptic ulcer of the antrum of the stomach in men is similar to that of duodenal ulcer. We have found this to be usually, but not invariably, true Uropepsin excretion in gastric ulcer or gastric cancer not localized in the antrum is usually in the normal or low range (4, 11, 21) Peptic ulcer in women is not accompanied by

increased uropepsin values (4, 21). It is also true that very high levels of uropepsin are encountered in the absence of ulcer symptoms.

A survey of gastric lesions was made at the Los Angeles County Hospital.* The preoperative uropepsin determinations in the patients with gastric carcinoma and the results of an 18 month follow-up of these patients after resection of the stomach are given in Table 1. It is noteworthy that in this small series the patients with resectable lesions who had very low uropepsin levels died relatively soon after surgery. Eastcott *et al.* (11) and others (1) have pointed out that possibly the low uropepsin values in some cases of gastric carcinoma represent

TABLE 1 – GASTRIC CARCINOMA (PATHOLOGIC DIAGNOSIS)

CONDITION OF CARCINOMA	CASES	PREOPERATIVE UROPEPSIN RANGE, UNITS		
		0–20	20–40	40+
Nonresectable	18	14	4	0
Resectable	9	6*	2	1

* All 6 resectable patients having a low or negative uropepsin output died within 1 year of surgery, 4 deaths were operative (within 1 month postoperatively) The 3 patients with a substantial uropepsin output are living, 2 of them now over 1 year postoperatively

inadequate protein nutrition as well as the destructive effect of the cancer itself. The unusually difficult postoperative course and early deaths in our group of patients might substantiate this view.

IN ACHLORHYDRIA AND PERNICIOUS ANEMIA

In any process in which there is destruction or degeneration of the gastric mucosa with resultant diminished peptic activity, the uropepsin excretion will decrease The conditions in which achlorhydria is pres-

[illegible]

other gastric lesions.

Uropepsin is not present in pernicious anemia (12) a finding that *has been repeatedly verified As a diagnostic aid in the clinical diagnosis* of pernicious anemia or achlorhydria, the uropepsin determination can indicate the state of secretory activity of the stomach.

* With the help of Drs I G. Macdonald and L W Guss

After Gastric Surgery

Except with total removal of the stomach, the stress reaction to the surgical procedure on the stomach or other operations or injuries causes a marked rise in uropepsin excretion during the first postoperative week (11, 18). After that, the uropepsin decreases to approximately preoperative levels. Many determinations on patients at the Los Angeles County Hospital who have had gastric resection have shown uropepsin values somewhat lower but approximately equivalent to those before surgery. In operations for duodenal ulcer we have been unable to evaluate the adequacy of the resection by uropepsin determinations. The excretion of uropepsin certainly is not proportional to the amount of gastric tissue remaining after surgery (4). High preoperative uropepsin levels drop to lower levels after resections for duodenal ulcer (4, 11), but the level after surgery may in part be influenced by the lessening of stress on the patient which follows the removal of the ulcer.

In patients with recurrent peptic ulceration following gastric resection or gastroenterostomy, in whom it is difficult to measure the gastric acid, the uropepsin determination is valuable in determining gastric activity.

Eastcott *et al.* have pointed out that immediately after gastric surgery uropepsin rises rapidly and is higher than following surgery in gallbladder disease. They feel that this might be due to mechanical manipulation of the stomach during the gastric resection. We, too, have found the rise following gastric resection to be more sudden and usually higher than the delayed response following other operations, seeming to suggest that mechanical manipulation of the stomach may cause sudden temporary uropepsin responses It may also mean that the uropepsin changes due to psychic stimulation may be caused merely by the neurogenic stimulation of gastric motility rather than by direct action of the nerve stimuli on the peptic activity.

During Hormone Therapy

Adrenocortical substances are now being used in the treatment of many disease entities. Since the introduction of these substances it is well known that peptic ulcer can be activated by large dosages of ACTH or cortisone Gray *et al* (18) have emphasized the need for the ulcer diet and administration of antacids during such therapy

In the collagen diseases which respond well to cortisone therapy, the uropepsin levels do not rise significantly except when the cortisone dosages are large (e.g, 150 mg. or more per day) (4, 26, 32). This would seem to indicate that the disease itself reduces the capacity of the gastric mucosa to respond normally to cortisone, or that the diseased tissue takes up cortisone preferentially so that there is no excess of cortisone to overstimulate normal tissue. *We have found it helpful* to measure the uropepsin excretion during cortisone therapy as a guide to the strictness to be observed in the ulcer program. Anticholinergic drugs do not lower the uropepsin level during cortisone therapy (4).

In conditions such as lymphoma, in which there is autolysis of the tumor in response to cortisone therapy, large dosages of cortisone must be used for short periods of time. West *et al* (36) report that maintained levels of uropepsin excretion of 100 to 150 units per hour (by their assay method) represent a cortisone dosage adequate to cause shrinkage of the tumor. In the absence of clinical response when the cortisone is administered until these levels of uropepsin are reached, larger doses are not effective, in their experience. With regard to these responses in lymphoma, we have found that uropepsin excretion was stimulated when the lymphoma involved the stomach. In 3 patients with gastric lesions, later proved to be lymphosarcoma, the uropepsin excretions were higher than normal; a fourth patient with gastric lymphosarcoma did not have elevated uropepsin levels.

We have not found an increase in uropepsin excretion with any other hormone therapy. Aside from Brown and Reifman's (7) report of a significant rise in uropepsin excretion in a few of their patients who were receiving testosterone, there is no report of such hormonal stimulation in the literature nor was there any increase in uropepsin excretion in our patients with lupus erythematosus who were on testosterone therapy.

DISCUSSION AND SUMMARY

The foregoing review of uropepsin excretion as a measure of peptic activity of the stomach may indicate the practical advantages of such measurement (*1*) Essentially the same information is obtained by this assay as by gastric analysis, with the additional advantage that it provides an estimate of the total, constant, functioning activity of the stomach (2) It is much easier to obtain the urine specimen than a specimen of gastric juice. (3) The simple milk coagulation test has

proved to be entirely adequate. (*4*) Data to date suggest that this test can provide valuable information with regard to the effect of emotional and hormonal stresses on the patient as well as information about the functioning state of the stomach and possibly the state of nutrition.

There is evidence that a direct effect of psychic stress in elevating uropepsin secretion can be separated from a stress effect which involves adrenocortical oversecretion and secondary gastric response to emotional tension. Patients in whom peptic ulcer is related primarily to psychic stress, that is, those who show a prompt rise in uropepsin excretion on emotional stress, may well be found to respond adequately to a vagotomy, if surgical intervention is indicated. In these patients subtotal gastric resection may be avoided. Further investigation of uropepsin excretion will surely lead to a better clinical understanding of the factors involved in peptic ulcer and its management.

The clinician must be cautious in his use of cortisone or corticotropic substances. Whereas adequate clinical response to treatment may not stimulate the stomach to hyperfunction, evidence of undesirable gastric stimulation, possibly due to overdosage, or requiring ulcer therapy can usually be obtained by uropepsin assay. This method is now being used for controlling treatment with high dosages of cortisone, specifically for therapy of tumors such as lymphosarcoma.

Not enough evidence is available to determine whether the uropepsin level is a useful aid in estimating the prognosis in patients with gastric cancer or whether it merely reflects the state of nutrition of a given patient However, this assay will be important in conjunction with the other technics being studied for the evaluation and treatment of these patients

REFERENCES

1 Adrenocorticotropic hormone and gastric secretory function, Nutrition Rev 8 309, 1950
2 Anson, M L, and Mirsky, A E The estimation of pepsin with hemoglobin, J Gen Physiol 16 59, 1932
3 Asher, L Personal communication
4 Balfour, D C, Jr Unpublished data
5 Balfour, D C, Jr, Preston F W, and Bolman, J L The disappearance of uropepsin following total gastrectomy, Gastroenterology 10 5, 880, 1948
6 Broh-Kahn, R H Podore, C J and Mirsky, I A Uropepsin excretion by man II Uropepsin excretion by healthy men, J Clin Invest 27 825, 1948
7 Brown, W H, and Reifman, A G Personal communication
8 Brucke, E Die verdauende Substanz im Urin, Sitzungsb d k Akad d

Wissensch, Math-naturw. Cl 43 618, 1861. [Cited by Farnsworth *et al* (11).]
9 Bucher, G. R: Uropepsin. A review of the literature and report of some experimental findings, Gastroenterology 8 627, 1947.
10 Bucher, G. R, and Anderson, A Uropepsin output in cats treated with caffeine and histamine, Am J. Physiol. 153 454, 1948
11 Eastcott, H. H. G, Fawcett, J K., and Rob, C. G Some factors affecting uropepsinogen excretion in man, Lancet 1.1068, 1953.
12 Farnsworth, E B; Speer, E, and Alt, H L The quantitative determination of a pepsin-like substance in the urine of normal individuals and of patients with pernicious anemia, J Lab & Clin Med 31 1025, 1946
13 Folin, A, and Ciocalteu, V On tyrosine and tryptophane determinations in proteins, J Biol Chem 73 627, 1927
14 Frouin, A · Sur l'origine et le lieu de résorption de la pepsine urinaire, Compt. rend Soc. de biol 56.204,1904.
15 Garst, J B., and Hilliard, J Unpublished data.
16 Goodman, R. D, Sandoval, E., and Halsted, J. A Observations on some of the technical and clinical factors influencing the determination of uropepsin excretion in man, J Lab & Clin Med 40 6, 872, 1952.
17 Gottlieb, E Untersuchungen uber die Propepsinmengen im Blut und Harn, Skand Arch f Physiol. 46 1, 1924
18 Gray, S, Jr, *et al*. The significance of hormonal factors in the pathogenesis of peptic ulcer, Gastroenterology 25 156, 1953
19 Halsted, J A Personal communication
20 Hilliard, J, *et al* Personal communication
21 Hirschowitz, B I Urinary excretion of pepsinogen in gastroduodenal ulceration, Lancet 1 66, 1953
22 Hollander, F Current views on the physiology of the gastric secretions, Am J Med 13 453, 1952
23 Jacobs, J S L, Tempereau, C E, and West, P M The effect of insulin coma on uropepsin excretion, Science 116 86, 1952
24 Janowitz, H D, and Hollander, F Relation of uropepsinogen excretion to gastric pepsin secretion in man, J Appl Physiol 4 53, 1951
25 Janowitz, H D, Levy, M H, and Hollander, F The diagnostic significance of urinary pepsinogen excretion in diseases of the upper gastrointestinal tract, Am J M Sc 220 679, 1950
26 Mendeloff, A I Personal communication
27 Mirsky, I A, Futterman, P, and Kaplan, S Blood plasma pepsinogen II The activity of the plasma from "normal" subject, patients with duodenal ulcer, and patients with pernicious anemia, J Lab & Clin Med 40 188, 1952
28 Mirsky, I A, Kaplan, S, and Broh-Kahn, R H Pepsinogen excretion (uropepsin) as an index of the influence of various life situations on gastric secretion, Proc A Res in Nerv & Ment Dis 29 628, 1950
29 Mirsky, I A, *et al* Uropepsin excretion by man I The source, properties and assay of uropepsin, J Clin Invest 27 818, 1948
30 Mirsky, I A, *et al* Blood plasma pepsinogen I The source, properties and

assay of the proteolytic activity of plasma at acid reactions, J. Lab & Clin Med. 40 17, 1952.

31. Fodore, C J., Broh-Kahn, R. H., and Mirsky, I. A: Uropepsin excretion by patients with peptic ulcer and other lesions of the stomach, *J Clin Invest* 27.834, 1948

32 Sachar, L A., and Sicher, N.. Uropepsin and urorennin, Bull Jewish Hosp, St Louis, p 29, May, 1953

33 Spiro, H M, Reifenstein, R. W, and Gray, S J: The effect of ACTH upon uropepsin excretion, *J Lab. & Clin Med* 35.899, 1950

34 Sylvest, O: The method for determining the renninogen of urine, Acta med. scandinav 133 289, 1949

35 West, P M.. *Personal communication*

36. West, P M, Ellis, F W, and Scott, B L A simplified method for determining the excretion rate of uropepsin, J Lab & Clin Med. 39 159, 1952

Glucagon, the Hyperglycemic-Glycogenolytic Hormone of the Pancreas*

PIERO P. FOÀ

The Chicago Medical School, Chicago

INSULIN HAD just been discovered when it was reported that, following its intravenous administration, the characteristic hypoglycemic effect was preceded by a brief, but significant, hyperglycemia (28, 58). This observation (Fig 1) was confirmed repeatedly and led to the discovery of a second pancreatic hormone, glucagon More than 300 reports have been published on this subject. Only some of them will be cited here, and the interested reader is referred to excellent reviews for further information (3, 14, 18, 36, 37, 53, 61, 70, 79, 81, 112, 131).†

When first observed, the "initial insulin hyperglycemia" was believed to be the result of a "paradoxic" glycogenolytic effect of insulin, and this belief gave rise to a controversy on the mechanism of action of this hormone which lasted until Abel and co-workers (1) succeeded in preparing a crystalline insulin free of this hyperglycemic action It then became apparent that the phenomenon was due not to insulin itself, but to a contaminant which remained in the mother liquor of crystallization (73) and which, to most investigators interested at the time in the production of pure insulin, remained nothing more than a

---

ing

"contaminant." Murlin and associates (87, 107) were the first to suggest that the contaminant might be a physiologic material produced by the pancreas, and proposed the name "glucagon," meaning "mobilizer of sugar," for it. Interest in glucagon, however, remained limited until 1934, when Scott (122) reported a practical method for the crystallization of insulin. Scott's method was soon adopted by most manufacturers although, unlike Abel's method, it did not remove the hyperglycemic material. Soon the "hyperglycemic-glycogenolytic effect" of insulin again was observed in several laboratories, giving rise to a re-

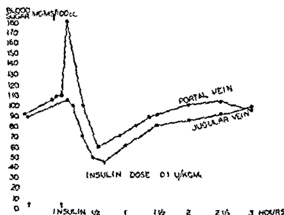


FIG. 1.—Blood sugar changes after intravenous injection of insulin (27)

newed controversy on the mechanism of insulin action and to a renewed interest in glucagon (13, 46, 55, 56, 97, 104, 123).

ORIGIN, DISTRIBUTION, AND CHEMICAL PROPERTIES OF GLUCAGON

Glucagon-like materials have been found not only in commercial insulin (36, 38, 41, 109, 134, 150) and in the perfusate and extracts of normal pancreas (46, 84, 135, 146), but also in the extract of the beta-cell-free pancreas of alloxan diabetic animals (24, 64, 71, 110, 119) and in the endocrine pancreas lacking acinar tissue of certain fish after the destruction of the beta cells with alloxan (99). Glucagon can be found in fetal pancreas before the acinar tissue becomes functional and in the remnants of adult pancreas after destruction of the beta cells with alloxan and after atrophy of the acinar tissue due to ligation of the ducts (24, 25, 64, 71, 110, 119) (Fig. 2).

These observations rule out the beta cells of the islets of Langerhans and the acinar tissue, and suggest the alpha cells as the most probable site of glucagon formation (*Fig.* 3). Glucagon can be extracted also from gastric mucosa containing alpha-like argentaffine cells similar to those of the pancreas (67, 92, 110, 134) (Fig. 4). Granules of secretion have been described in the cytoplasm of alpha cells. In addition, certain compounds, such as diethyldithiocarbamate and cobaltous chloride, which seem to cause selective damage to the alpha

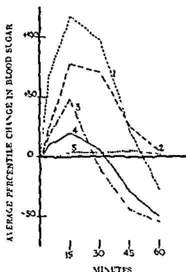


FIG 2.—Average blood sugar changes in 10 kg dogs after intravenous injection of extracts prepared from 6.5 to 7.0 Gm of tissue (110) *1*, fetal calf pancreas, *2*, pancreas from alloxan-treated dogs, *3*, dog pancreas following duct ligation, *4*, normal dog pancreas, *5*, parotid gland of dog and cattle

cells (6, 82, 83, 102, 139, 139a, 140) (Fig 5), reduce the amount of glucagon present in the pancreas to about one-third of normal (143). For these reasons, glucagon has been called the "alpha cell hormone" (77).

This hypothesis, although not universally accepted (42, 93, 141), offers a possible explanation for the high insulin requirement of the alloxan diabetic animal as compared to the depancreatized one The alloxan diabetic animal, having no beta cells, but with its alpha cells intact, would be deprived of insulin but not of blood sugar-raising glucagon In contrast, the depancreatized animal would require less

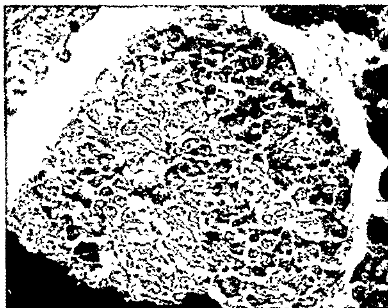


FIG 3.—Microphotograph of an islet of Langerhans of the guinea pig, showing alpha cells (light) and beta cells (dark). Gomori's stain; ×630.

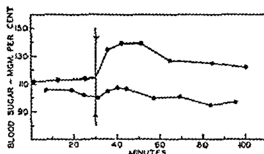


FIG 4.—Blood sugar changes after intravenous injection of extracts of dog gastric mucosa into normal rabbits (134) Upper curve, injection of an extract from 5 Gm of mucosa, lower curve, from 1 0 Gm

insulin because neither hormone is present Not only is the insulin requirement of depancreatized animals and men relatively low (35, 42, 72, 74), but pancreatectomy reduces the insulin requirement of alloxan diabetic animals (116, 136), of animals rendered diabetic by means of anterior pituitary extracts (100), and of diabetic man (33, 115). Although in some instances this reduction may be due to de-

creased food absorption after removal of the pancreas (105), in other experiments this possibility has been ruled out (23, 100, 117), and the lack of glucagon appears to be the most probable explanation of the observed facts

Glucagon-like materials, perhaps products of its metabolic breakdown, have been found also in normal urine (101, 151), and in the

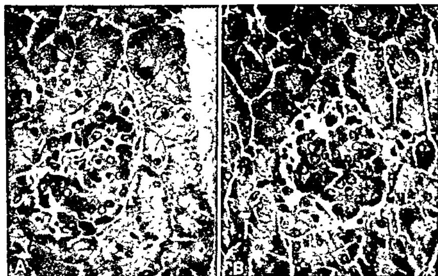


FIG. 5—Islet of Langerhans from a rabbit that died of hypoglycemia about 20 [illegible] G [illegible] p [illegible] a [illegible] degeneration, such as pyknosis of nuclei and disintegration of cytoplasm

urine of diabetic patients and of patients suffering from oneirophrenia* (103, 106), and of alloxan diabetic animals (121).

Several attempts to prepare pure glucagon have been made Insulin-free glucagon was first obtained by Sopp (127) in Burger's laboratory. It was found to be a nondialyzable, protein-like material which could be purified by precipitation with picric or trichloracetic acid, ether, acetone, and alcohol, and to have elementary composition and chemical properties resembling those of insulin. Like insulin and other

* A form of schizophrenia characterized by illusions, hallucinations, amnesia, and stupor.

proteins, glucagon is soluble in liquid ammonia and can be extracted with it directly from dehydrated pancreas or gastric mucosa (67) (Fig. 6). Glucagon is more resistant than insulin to alkali (135) and to the reducing action of cysteine. However, it is less resistant to the action of trypsin (138). Glucagon has a characteristic electrophoretic peak (135). Using these and other properties, glucagon was purified (39, 40) and, recently, crystallized by Staub *et al.* (128, 129) (Fig 7). These workers showed that glucagon is a protein

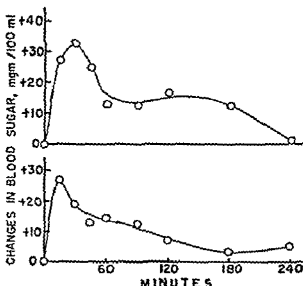


FIG. 6 – Blood sugar changes in rabbit after intravenous injection of liquid ammonia extract of pancreas (upper curve) and of stomach (lower curve) (67)

having a probable molecular weight of 6,000 to 8,000, that it contains all of the amino acids present in the insulin molecule except proline, isoleucine, and cystine, and two amino acids (methionine and tryptophane) not present in insulin Unlike crystalline insulin, crystalline glucagon contains practically no zinc Staub and associates have proposed that 1 unit of glucagon be defined as the amount of glucagon having the hyperglycemic effect of 0 1 μg. of the crystalline material, and have shown that one-half this amount per kilogram of body weight is sufficient to cause a significant hyperglycemia in the anesthetized cat Since 0 1 μg of crystalline insulin contains only 0 0025 unit and since at least 0 025 unit of insulin per kilogram of body weight is necessary to cause measurable hypo-

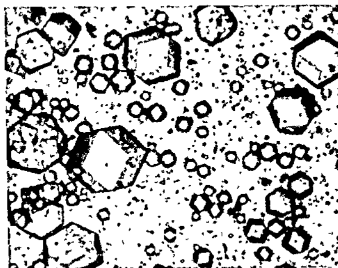


FIG 7—Glucagon crystals (128)

glycemia, it becomes apparent that, on a weight basis, the biologic potency of glucagon is at least 10 times greater than that of insulin.

PHYSIOLOGIC PROPERTIES AND HORMONAL NATURE OF GLUCAGON

The physiologic properties of glucagon have been investigated extensively Burger and his collaborators (15–20) have demonstrated that the hyperglycemic effect of glucagon injected subcutaneously or intramuscularly is not as marked as when it is injected intravenously, that the effect is greater when glucagon is injected into the portal vein than when it is injected into a systemic vein and that glucagon has no effect when the circulation to the liver has been occluded Similar observations were made by Collens and Murlin (27) and later confirmed by others in several animal species, including man (5, 84, 110) (Figs. 8 and 9) Burger *et al* also observed that the intensity of the hyperglycemic response depends on the amounts of liver glycogen available and is decreased when the glycogen reserves are low, as in pro-

that glucagon stimulates the phosphorylase system and, therefore, the breakdown of glycogen to glucose-l-phosphate (Fig. 10). Recently, by means of venous catheterization, Kibler and Myers (86) found that the continuous infusion of glucagon in man causes a marked increase in glucose production by the liver.

An indirect demonstration of the effect of glucagon on liver glycogen was furnished by Weisberg and Kaplan (147, 148), who found that the administration of cobalt chloride to rats causes an immediate

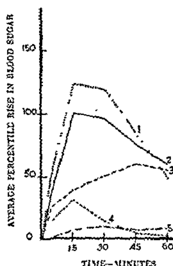
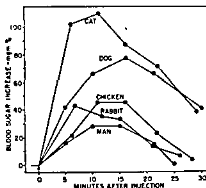


FIG. 8.—Average blood sugar rise after injection of inactivated insulin by different routes (110) *1*, intraportally, *2*, intravenously, *3*, intraperitoneally, *4*, intramuscularly, *5*, subcutaneously

decrease in liver glycogen, which seems to occur simultaneously with a rise in blood sugar level, and which is followed 24 hours later by a significant increase in liver glycogen and a tendency to hypoglycemia (139–141) They suggested that destruction of the alpha cells by cobalt releases preformed glucagon, causing the initial glycogenolysis, when the reserve of glucagon is exhausted, no more is formed, the rate of glycogenolysis is reduced, and glycogen is allowed to accumulate in the liver. This sequence of events is analogous to that which follows the destruction of the beta cells by alloxan or other compounds, when the liberation of preformed insulin causes an immediate hypoglycemia followed by a permanent diabetes (Fig. 11).

The depletion of liver glycogen may be responsible for the increase



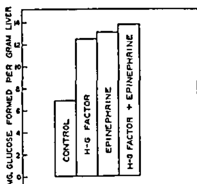


FIG. 9 (*left*) —Effect of glucagon (hyperglycemic factor, HGF) in various species, 0.05 mg of glucagon was administered intravenously after induction of anesthesia (96).

FIG. 10 (*right*) —Effect of a maximal amount of glucagon (H-G factor) and epinephrine, added separately and together, on the glucose output from liver slices (131).

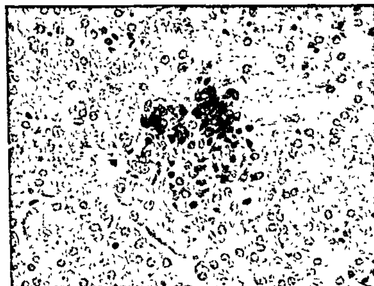


FIG. 11 —Islet of Langerhans of rabbit killed 5 days after intravenous injection of dithizone, note reduced size of islet and that it is composed chiefly of alpha cells with a few pyknotic nuclei (82). Gomori's chromium-hematoxylin phloxine stain

in concentration of ketone bodies in the blood (62), and in ketone body production by liver slices (130) observed following the administration of glucagon. Glucagon seems to influence the glycogen content of skeletal and heart muscle, but this effect has not been fully elucidated and may depend on the amount of glucose liberated from the liver (113, 148). Glucagon has little or no effect on blood lactic acid and does not affect blood pressure Thus, unlike epinephrine, which also promotes liver glycogenolysis, glucagon elevates blood

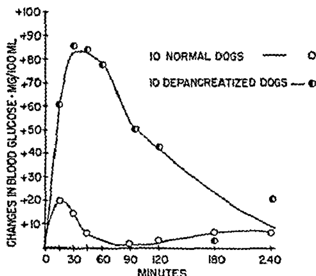


FIG 12.–Effect of glucagon on the blood sugar of normal and of depancreatized dogs controlled with insulin (61)

sugar without other measurable effects (144), even after the administration of sympathetic blocking agents (42, 43).

Experiments done in our laboratory and elsewhere have shown that the hyperglycemic action of glucagon is much greater in the depancreatized dog controlled with insulin than in the normal animal (65, 110). Similarly, insulin produces more marked hypoglycemia in the depancreatized dog than in the normal dog (26, 65) Thus, the controlled diabetic dog appears to be more sensitive than the normal dog to the action of both glucagon and insulin (Figs 12 and 13) On the other hand, when the diabetic animal is deprived of insulin and ketosis develops, the response to glucagon decreases as the concentration of ketone bodies in the blood increases (Fig 14) Probably both phe-

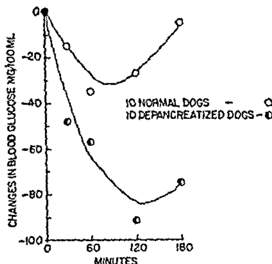


FIG. 13.—*Effect of insulin on the blood sugar of normal and of depancreatized dogs controlled with insulin* (61).

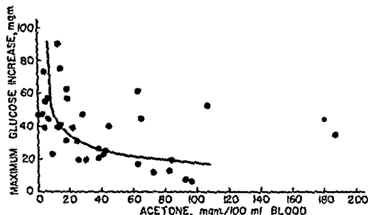


FIG. 14.—*Relation between hyperglycemic effect of glucagon and concentration of acetone in the blood of noncontrolled depancreatized dogs* (61).

nomena are the result of the progressive decrease in liver glycogen occurring in the decompensated diabetes of the dog. A possible explanation for the relatively low sensitivity of the normal animal to glucagon is that the hyperglycemia produced by the injection of glucagon may stimulate insulin secretion in the same way as the hyperglycemia produced by the injection of glucose (2, 64, 154–154b). In

the presence of a normal pancreas, the effect of glucagon would be automatically limited, and only in the depancreatized animal, with a good supply of liver glycogen, could glucagon exert its full action. The *observation that insulin hypoglycemia is greater in the depancreatized than in the normal animal agrees* with the results of other workers (4, 11, 26), and suggests that, in the normal animal, there may be a reciprocal relationship between the secretion of insulin and the secretion of glucagon. Thus, in the normal animal, insulin hypoglycemia may cause the secretion of glucagon and, in turn, be limited by

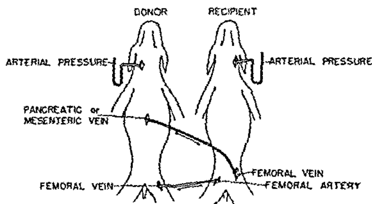


Fig 15 – Cross-circulation set-up The pancreatic (or *duodenal*) blood of donor flows into the femoral vein of recipient, a return flow is established between femoral artery of recipient and femoral vein of donor, the pancreatic-femoral anastomosis is open continuously, while the return flow is open only as necessary to keep the blood pressure of donor and recipient at approximately their original level The dogs are heparinized (61)

it, on the other hand, in the depancreatized animal, insulin would be free to show its full effect

*The evidence presented in the preceding paragraphs strongly in*dicates that glucagon *is a second* pancreatic hormone, a hypothesis suggested by Burger in 1937 (17), but questioned by others Waters (145), for example, referred to glucagon as a "hormone presumptive," while Conn (30), as late as 1953, stated that the "so-called alpha cell hormone may yet prove to be an artifact rather than a normally produced physiologic substance."

The demonstration that glucagon is secreted into the blood stream would constitute strong and direct evidence of its hormonal nature (37) This was suggested by observations made in our laboratory on

alloxan diabetic dogs (64), and confirmed in normal animals by means of cross-circulation experiments. In these experiments, the pancreato-duodenal vein of a donor dog (*D*) was anastomosed with the femoral vein of a recipient dog (*R*), and a femoral artery of dog *R* was anastomosed with a femoral vein of dog *D* (Fig. 15). In this preparation, pancreatic blood of dog *D* flows into dog *R*, and systemic blood of dog

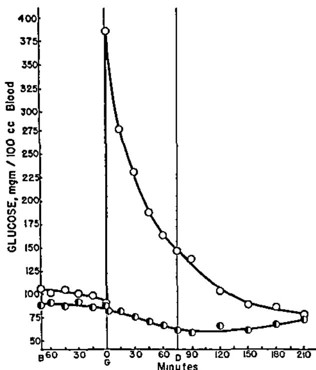


FIG. 16.—Pancreatic-femoral anastomosis (64) *B*, before anastomosis, *G*, glucose injected into donor (open circles), *D*, dogs disconnected.

R flows back into dog *D*, restoring its blood volume and maintaining the blood pressure of both animals at approximately normal levels. With this technic, the secretion of insulin or glucagon by the pancreas of dog *D* can be detected by the change produced in the blood sugar of dog *R* (59, 64). Thus it was possible to demonstrate that when glucose is injected into dog *D*, the rise in its blood sugar level is followed by sharp hypoglycemia in dog *R*, suggesting that the injection of glucose stimulates the pancreas of the donor to produce insulin (Fig 16)

the presence of a normal pancreas, the effect of glucagon would be automatically limited, and only in the depancreatized animal, with a good supply of liver glycogen, could glucagon exert its full action. The observation that insulin hypoglycemia is greater in the depancreatized than in the normal animal agrees with the results of other workers (4, 11, 26), and suggests that, in the normal animal, there may be a reciprocal relationship between the secretion of insulin and the secretion of glucagon. Thus, in the normal animal, insulin hypoglycemia may cause the secretion of glucagon and, in turn, be limited by

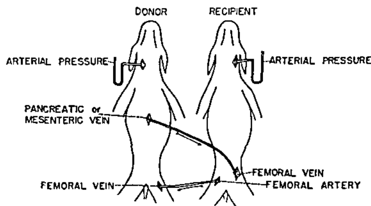


FIG 15—Cross-circulation set-up. The pancreatic (or duodenal) blood of donor flows into the femoral vein of recipient, a return flow is established between femoral artery of recipient and femoral vein of donor; the pancreatic-femoral anastomosis is open continuously, while the return flow is open only as necessary to keep the blood pressure of donor and recipient at approximately their original level The dogs are heparinized (61)

it, on the other hand, in the depancreatized animal, insulin would be free to show its full effect.

The evidence presented in the preceding paragraphs strongly indicates that glucagon is a second pancreatic hormone, a hypothesis suggested by Burger in 1937 (17), but questioned by others. Waters (145), for example, referred to glucagon as a "hormone presumptive," while Conn (30), as late as 1953, stated that the "so-called alpha cell hormone may yet prove to be an artifact rather than a normally produced physiologic substance."

The demonstration that glucagon is secreted into the blood stream would constitute strong and direct evidence of its hormonal nature (37) This was suggested by observations made in our laboratory on

in turn causes hyperglycemia in the recipient. When the dogs are connected through a mesenteric-femoral anastomosis, the injection of insulin into the donor is followed by a decrease in the blood sugar of the recipient, presumably because the mesenteric blood contains some of the injected insulin, but no glucagon.

The hypothesis that glucagon is a true hormone has received support from these experiments. The secretion of this hormone, like that of insulin, appears to regulate and, in turn, be regulated by the concentration of glucose in the blood. In addition, through their effect on

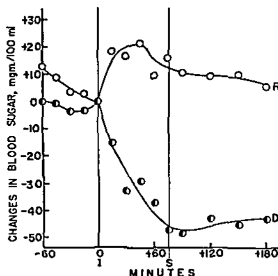


FIG. 18.—Pancreatic-femoral anastomosis (66). *I*, injection of insulin into donor, other symbols as in Figure 17

blood sugar, insulin and glucagon appear to regulate each other. Thus, when the secretion of glucagon tends to raise the blood sugar level, insulin is secreted and lowers it, when too much insulin is secreted (or injected), its hypoglycemic effect is limited by the secretion of glucagon.

Glucagon, like epinephrine, should be considered a physiologic anti-insulin hormone, promoting the release of glucose from the liver and thus constituting a prompt compensatory response to insulin hypoglycemia The anti-insulin hormones of the anterior pituitary and the adrenal cortex also are activated by hypoglycemia to promote the formation of glucose from noncarbohydrate sources, raise the blood

This insulin is then carried by the anastomosis into the recipient, where it causes a fall in blood sugar level. When, in control experiments, the dogs are connected by means of a mesenteric-femoral anastomosis, the injection of glucose into the donor dog is followed by a rise in the blood sugar of the recipient because the mesenteric blood of the donor contains glucose, but no insulin. Similarly, when the pancreatic blood is derived from an alloxan diabetic donor, the injection of glucose into the donor is followed by a rise in the blood sugar of the recipient, as the pancreatic blood contains glucose and possibly glucagon, but no insulin. The injection of saline fails to produce sig-

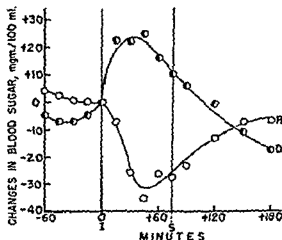


FIG 17.—Pancreatic-femoral anastomosis (66) *I*, injection of glucagon into donor (*D*), *S*, animals separated, *R*, recipient dog

nificant changes in the blood sugar of either dog The results indicate that the hypoglycemia observed in the recipient dog following the injection of glucose into the donor is the result of an increased insulin secretion brought about by the hyperglycemia Hyperglycemia can be produced in dog *D* by the injection of glucagon instead of glucose This hyperglycemia is followed also by a sharp decline in the blood sugar of dog *R*, suggesting that glucagon, like glucose, stimulates the pancreas of the donor to produce insulin (Fig 17). On the other hand, when hypoglycemia is produced in the donor by the injection of insulin, the blood sugar of the recipient increases (Fig 18) This suggests that insulin hypoglycemia in the donor causes the secretion of glucagon which, carried through the pancreatic-femoral anastomosis,

of Langerhans, but leaves the alpha cells intact (7, 34, 76, 88). (4) Radioactive growth hormone tends to concentrate in the pancreas (126). (5) Seemingly insulin-free glucagon preparations cause changes in the epiphyseal cartilage of hypophysectomized rats identical to those induced by growth hormone (44). (6) A hyperglycemic material can be found in the pancreatic, but not in the femoral, blood of rats treated with anterior pituitary growth hormone (12, 152). Cross-circulation experiments similar to those already described (68) showed that when anterior pituitary growth hormone (somatotropic hormone or STH) is injected intravenously into a normal donor dog, the blood sugar level of the donor rises and that, simultaneously, a hyperglycemic material, presumably glucagon, appears in the blood of the pancreatic vein and causes hyperglycemia in a normal recipient (Fig. 19). No hyperglycemic material can be detected in the blood of the mesenteric vein These results are in agreement with those of Carballeira and associates (22), who observed a rapid hyperglycemic effect following a single intravenous injection of STH in man, and with those of Myers and co-workers (108), who found a sharp increase of blood sugar in both the hepatic vein and the femoral artery of man 15 minutes after an intravenous injection of STH or glucagon. Thus, the diabetogenic effect of crude anterior pituitary extracts (29, 80), and of purified growth hormone (21, 32, 34) may be due, in part, to the secretion of glucagon.

CLINICAL SIGNIFICANCE OF GLUCAGON

Much evidence points to the possibility that glucagon may play an important role in clinical medicine and perhaps in therapy. For instance, it has been suggested that an excessive secretion of glucagon may exist in diabetes mellitus Ferner (51–54) found that the pancreas of patients with diabetes not only shows signs of decreased beta-cell activity, as previously shown, but also contains an increased number of alpha cells (Fig. 20). On the basis of this histologic evidence, Ferner believed that, in diabetes, insulin insufficiency is associated with an increased production of glucagon Ferner's histologic observations have been confirmed by Gomori (75) and by Hess (78). Hess examined 2,000 islets of Langerhans in the pancreas of 10 normal individuals and of 10 diabetic patients and found that in normal individuals the average ratio of beta cells to alpha cells varies between 15 1 and 5:1, whereas in diabetic patients the ratio varies between 6.1 and 2.1. This absolute and relative increase in the number of alpha

sugar to normal (45), and even cause compensatory hyperglycemic tides (124, 125). According to Somogyi (125), this may lead the unaware physician to increase the already excessive insulin dosage further and enter a vicious cycle of overtreatment. By stimulating the secretion of glucagon and epinephrine, insulin may cause a depletion of liver glycogen and may appear to have a "diabetogenic effect" (95). The powerful anti-insulin action of glucagon in the intact animal has been confirmed by the experiments of Tyberghein (137), who was able

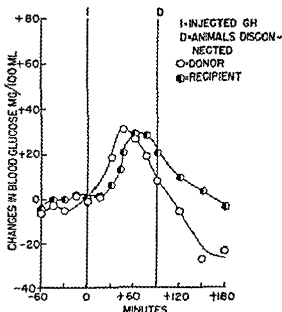


FIG 19 – Pancreatic-femoral anastomosis (68).

to maintain normal blood sugar values in heavily insulinized rabbits by continuous infusion of glucagon

It has been suggested that the secretion of glucagon may be stimulated not only by hypoglycemia, but also by a hormone of the anterior pituitary (85) This hypothesis is based on the following observations (*1*) The pancreas of children during the period of growth, when presumably the anterior pituitary growth hormone is produced in great quantities, seems to be especially rich in alpha cells (50) (*2*) A hyperglycemic material found in the urine of normal animals disappears after hypophysectomy (151) (*3*) Diabetogenic growth hormone causes destruction of the insulin-secreting beta cells of the islets

of Langerhans, but leaves the alpha cells intact (7, 34, 76, 88). (4) Radioactive growth hormone tends to concentrate in the pancreas (126). (5) Seemingly insulin-free glucagon preparations cause changes in the epiphyseal cartilage of hypophysectomized rats identical to those induced by growth hormone (44). (6) A hyperglycemic material can be found in the pancreatic, but not in the femoral, blood of rats treated with anterior pituitary growth hormone (12, 152). Cross-circulation experiments similar to those already described (68) showed that when anterior pituitary growth hormone (somatotropic hormone or STH) is injected intravenously into a normal donor dog, the blood sugar level of the donor rises and that, simultaneously, a hyperglycemic material, presumably glucagon, appears in the blood of the pancreatic vein and causes hyperglycemia in a normal recipient (Fig. 19). No hyperglycemic material can be detected in the blood of the mesenteric vein. These results are in agreement with those of Carballeira and associat[illegible] effect following a single [illegible] ith those of Myers and co-workers (108), who found a sharp increase of blood sugar in both the hepatic vein and the femoral artery of man 15 minutes after an intravenous injection of STH or glucagon. Thus, the diabetogenic effect of crude anterior pituitary extracts (29, 80), and of purified growth hormone (21, 32, 34) may be due, in part, to the secretion of glucagon.

CLINICAL SIGNIFICANCE OF GLUCAGON

Much evidence points to the possibility that glucagon may play an important role in clinical medicine and perhaps in therapy. For instance, it has been suggested that an excessive secretion of glucagon may exist in diabetes mellitus. Ferner (51–54) found that the pancreas of patients with diabetes not only shows signs of decreased beta-cell activity, as previously shown, but also contains an increased number of alpha cells (Fig 20). On the basis of this histologic evidence, Ferner believed that, in diabetes, insulin insufficiency is associated with an increased production of glucagon. Ferner's histologic observations have been confirmed by Gomori (75) and by Hess (78). Hess examined 2,000 islets of Langerhans in the pancreas of 10 normal individuals and of 10 diabetic patients and found that in normal individuals the average ratio of beta cells to alpha cells varies between 15:1 and 5:1, whereas in diabetic patients the ratio varies between 6:1 and 2:1 This absolute and relative increase in the number of alpha

cells may explain the presence of a material similar to glucagon in the urine of patients with diabetes mellitus (121).

In view of the hypothesis that excessive production of glucagon plays a role in diabetes, and of earlier observations that cobalt increases the hypoglycemic effect of insulin (8–10, 90), cobalt salts have been administered to patients in an attempt to destroy the alpha cells While some authors reported an improvement of the diabetic



Fig 20.—Cellular composition of the islets of Langerhans in the normal (*A*) and diabetic (*B*) pancreas of man, note prevalence of alpha cells (black) in diabetic pancreas (52)

syndrome (9, 94, 114), others obtained negative results (91), the therapeutic value of cobalt in diabetes mellitus, if any, therefore remains to be proved (153)

Pincus (111) made the interesting observation that patients with labile or brittle diabetes tend to show a greater hyperglycemic response to glucagon than normal subjects or patients with stable diabetes. He suggested that the pancreas of patients with labile diabetes may be deficient in alpha cells and therefore accumulates liver glycogen which then could be mobilized by glucagon On the other hand, Kirtley *et al* (69) found that patients with labile diabetes are less sensitive to glucagon than normal subjects or patients with stable dia-

betes, and suggested that labile diabetics may have less circulating insulin and therefore relatively low hepatic glycogen stores. They suggested further that the greater insulin resistance of the stable diabetic as compared to the labile diabetic may be due to the ability to counteract hypoglycemia with prompt secretion of glucagon and mobilization of the relatively abundant glycogen reserves. In this manner, the stable diabetic would resemble the alloxanized animal, while the unstable diabetic, having low glycogen reserves and little endogenous glucagon, would resemble the depancreatized animal This hypothesis can only be accepted with reservations, for in diabetes in man the liver is not poor in glycogen, even in patients who died in diabetic coma.

Dana *et al.* (31) reported that in patients with the Kimmelstiel-Wilson syndrome ketone bodies rarely accumulate sufficiently for acidosis to develop, they suggested that the hyperglycemia might be due to a relative excess of glucagon in the presence of enough endogenous insulin to prevent acidosis, while the retinal and renal capillary lesions would be due to a specific metabolic defect, as yet unexplained Perhaps the pancreas of these patients is still capable of producing not only glucagon, but also the "antiketosis factor" possibly present in insulin-free pancreatic extracts (62, 63, 130). It is also possible that the vascular lesions may be the result of years of inadequate insulin control associated with an elevation of low-density serum lipoproteins. Recent experiments (49) have shown that even small degrees of insulin insufficiency, with minimal glycosuria and without ketosis, can cause the accumulation of these lipoproteins in the serum of depancreatized dogs. An excessive secretion of glucagon may be present in patients with carcinoid tumors rich in alpha-like argentaffine cells (149), and in patients with oneirophrenia (103, 106) These patients tend to be insulin-resistant and to have a decreased tolerance for carbohydrates, as rats may have after receiving daily injections of glucagon for several months (69)

Glucagon deficiency in man may also occur Thus, McQuarrie *et al* (98) described a number of infants with persistent hypoglycemia and noted that their pancreas contained few alpha cells An insufficient secretion of glucagon may be responsible for the accumulation of liver glycogen in patients with glycogen storage disease (47, 112).

The possible role played by glucagon in insulin resistance has not been investigated, nor have the effects of glucagon impurities on insulin assay and on its action *in vitro* (5, 118, 132, 142) and *in vivo*

cells may explain the presence of a material similar to glucagon in the urine of patients with diabetes mellitus (121).

In view of the hypothesis that excessive production of glucagon plays a role in diabetes, and of earlier observations that cobalt increases the hypoglycemic effect of insulin (8–10, 90), cobalt salts have been administered to patients in an attempt to destroy the alpha cells. While some authors reported an improvement of the diabetic



FIG 20.—Cellular composition of the islets of Langerhans in the normal (*A*) and diabetic (*B*) pancreas of man, note prevalence of alpha cells (black) in diabetic pancreas (52)

syndrome (9, 94, 114), others obtained negative results (91), the therapeutic value of cobalt in diabetes mellitus, if any, therefore remains to be proved (153)

Pincus (111) made the interesting observation that patients with labile or brittle diabetes tend to show a greater hyperglycemic response to glucagon than normal subjects or patients with stable diabetes. He suggested that the pancreas of patients with labile diabetes may be deficient in alpha cells and therefore accumulates liver glycogen which then could be mobilized by glucagon On the other hand, Kirtley *et al* (89) found that patients with labile diabetes are less sensitive to glucagon than normal subjects or patients with stable dia-

these possible alternatives. Glucagon has a marked and unusually prolonged effect (up to 20 hours) in the alligator (155), perhaps because a low endogenous glucagon production allows the accumulation of large liver glycogen reserves in cold blooded animals. This hypothesis is suggested by the observation that the pancreas of hibernating animals is very poor in alpha cells, although rich in beta cells (160). The hypothesis that the secretion of glucagon is stimulated by anterior pituitary growth hormone finds new support in the results of Mayer and collaborators (102, 162) in mice with the obese-hyperglycemic syndrome and of Malandra and collaborators (161) in rats with alloxan diabetes. Elrick's (44) results have not been confirmed, and pure, insulin-free glucagon was found to have no growth-promoting effect (158). Ferner's hypothesis on the role of the alpha cells in the etiology of human diabetes was again presented and discussed (157), and the whole subject of glucagon reviewed (163).

Evidence that the alpha cells may not be the site of origin of glucagon has been reviewed (164). Although this view is contrary to strong physiologic evidence, it must be pointed out that many of the histologic studies cited in this review, in particular most of the work of Ferner and of Hess, have been done using silver staining methods of uncertain specificity The problem of the origin of glucagon and of its role in diabetes cannot be considered solved at the present time. Protracted administration of glucagon to rats causes infiltration of the liver and other changes probably not specific (165) The alpha cell-stimulating (alphacytotrophic) action of growth hormone has been confirmed both in the normal (166, 167) and in the hypophysectomized (168) animal This is in agreement with the results obtained by Foà *et al* (68) by means of cross-circulation experiments. MacGrath and Snedecor (169) found that insulin administration causes an increase in the glucagon content of the pancreas, confirming the finding of Foà *et al* (66) that insulin hypoglycemia stimulates glucagon production. Haugaard and Haugaard found that glucagon inhibits the synthesis of fatty acids and increases ketone body production by rat liver slices (170), confirming the in vivo observations of Foà and Weinstein (68).

REFERENCES

1 Abel, J J., *et al* Crystalline insulin, J Pharmacol, & Exper Therap 31 65, 1927
2 Anderson, E, and Long, J A The effect of hyperglycemia on insulin

(120). These effects may indeed be significant, as glucagon represents about 1 per cent of the protein material in crystalline insulin and is many times more active than insulin on a weight basis (41, 128, 129). The hyperglycemic response to an intravenous infusion of glucagon is smaller in patients with hepatitis and cirrhosis than in normal subjects and has been suggested as a test of liver function. The therapeutic possibilities of glucagon in liver storage disease, in hypoglycemia with or without islet cell tumor, and possibly other conditions, have not been investigated, although the use of other insulin antagonistic hormones (ACTH and cortisone) has been advocated in certain forms of encephalopathy due to hyperinsulinism (57).

CONCLUSION

Glucagon is a potent, quick-acting, anti-insulin hormone capable of raising the blood sugar concentration by promoting glycogenolysis in the liver. Glucagon appears to be secreted by the alpha cells of the islets of Langerhans in response to hypoglycemia and to stimulation by growth (diabetogenic) hormone of the anterior pituitary gland; like epinephrine and the anti-insulin hormones of the anterior pituitary and the adrenal cortex, glucagon is an important physiologic factor in the maintenance of a normal blood sugar concentration Although experimental and clinical observations suggest that glucagon may play a role in diabetes mellitus and other clinical disorders, the clinical, and possibly the therapeutic, significance of glucagon cannot be evaluated at the present time.

ADDENDUM

Since this review was completed, several papers on glucagon have appeared Ingle and collaborators (159) found that glucagon has no effect in the eviscerated rat, confirming the fact that the liver is the main site of action of this hormone. Ellis and collaborators (156) reported that the adrenergic blocking drug dihydroergotamine prevents hyperglycemia due to epinephrine and to cobaltous chloride, but not glucagon hyperglycemia Since cobaltous chloride is believed to destroy selectively the alpha cells, these results may indicate either that this destruction is not adequate to liberate sufficient amounts of glucagon or that the alpha cells are not the site of glucagon formation Since these authors do not offer histologic evidence of alpha-cell destruction in their cobalt-treated animals, one cannot choose between

22 Carballeira, A., *et al.*. Effects of single intravenous injections of pituitary growth hormone in normal adult men, Proc. Soc. Exper. Biol & Med 81.15, 1952.
23 Cavallero, C., and Malandra, B.. Effetto della pancreasectomia subtotale sul diabete allossanico del ratto, Boll Soc. ital. biol. sper. 25.85, 1949
24 Cavallero, C., and Malandra, B: Sull'effetto diabetogeno dei trapianti di pancreas allossanato nel ratto, Boll. Soc. ital. biol sper. 25.87, 1949
25 Cavallero, C., and Malandra, B.: Ulteriori ricerche sul fattore iperglicemizzante delle isole pancreatiche, Boll. Soc ital biol sper. 27:1221, 1951.
26 Collens, W. S., and Grayzel, H G Blood sugar response to intravenous insulin in normals and in diabetics, Proc Soc Exper Biol & Med. 28 487, 1931.
27 Collens, W. S., and Murlin, J R: Hyperglycemia following the portal injection of insulin, Proc. Soc Exper Biol & Med 26 485, 1929
28 Collip, J B· Delayed manifestation of the physiological effects of insulin following administration of certain pancreatic extracts, Am J Physiol 63 391, 1923
29. Conn, J. W. Diabetes mellitus and hyperinsulinism. Is there a common pathogenesis? Proc Am. Diabetes A 5·79, 1945.
30. Conn, J W· Endocrine regulation of the blood sugar, Ann Int. Med 38 179, 1953
31 Dana, G W., Eversole, S L., and Zubrod, C G Hyperglycemia and its relationship to diabetic retinitis and glomerular nodules, Bull Johns Hopkins Hosp 90 98, 1952.
32 de Bodo, R C., and Sinkoff, M W The role of growth hormone in carbohydrate metabolism, Ann New York Acad. Sc 57 23, 1953
33 Dixon, C F., *et al* Total pancreatectomy for carcinoma of pancreas in the diabetic person, metabolic studies, Arch Surg 52 619, 1946
34 Dohan, F C., Fish, C A., and Lukens, F D W Induction and course of permanent diabetes produced by anterior pituitary extracts, Endocrinology 28 341, 1941
35 Dragstedt, L R., Allen, J C., and Smith, E M Extensive insulin tolerance in diabetic dogs, Proc Soc Exper Biol. & Med 54.292, 1943.
36 de Duve, C *Glucose, Insuline et Diabète* (Bruxelles Goemaere, 1945)
37 de Duve, C Glucagon The hyperglycaemic-glycogenolytic factor of the pancreas, Lancet 2 99, 1953
38 de Duve, C., and Hers, H G La prétendue action glycogénolytique de l'insuline, Compt rend Soc biol 141 1147, 1947
39 de Duve, C., and Vuylsteke, C A Purification du glucagon (facteur hyperglycémiant-glycogénolytique du pancréas), Arch internat physiol. 61 107, 1953
40 de Duve, C., and Vuylsteke, C A The assay and purification of glucagon Proc Internat Physiol Congress (19th), Montreal, 1953, p 304
41. de Duve, C Hers, H G., and Bouckaert, J P Nouvelles recherches concernant l'action de l'insuline VI. Action de l'insuline sur le glycogène du foie, Arch internat pharmacodyn 72 45, 1946
42 Ellis, S., Anderson, H L., Jr., and Collins, M C Pharmacologic differentiation between epinephrine and HGF-hyperglycemias Application in the

secretion as determined with the isolated rat pancreas in a perfusion apparatus, Endocrinology 40 92, 1947.
3. Anderson, E., and Long, J. A.: The hormonal influences on the secretion of insulin, Recent Progress in Hormone Res 2 209, 1948
4 Anderson, E., Lindner, E., and Sutton, V.: A sensitive method for the assay of insulin in blood, Am J. Physiol 149 350, 1947.
5 Audy, G, and Kerly, M The content of glycogenolytic factor in some insulin samples, Biochem. J. 52 70, 1952, *see also* 52:77, 1952.
6 Avezzù, G., and Luise, R: Azione del cloruro di cobalto nel diabete allossanico del coniglio, Boll. Soc. ital biol sper 27:1515, 1951.
7. Bell, E T.. The incidence and significance of degranulation of the beta cells in the islet of Langerhans in diabetes mellitus, Diabetes 2:125, 1953.
8 Bertrand, G, and Mâcheboeuf, M. Influence du nickel et du cobalt sur l'action exercée par l'insuline, Compt rend Acad. sc. 182 1504, 1926, 183 5, 1926
9 Bertrand, G, and Mâcheboeuf, M · Nickel, cobalt et diabète, Compt. rend. Acad sc 183·257, 1926.
10 Bertrand, G, and Nakamura, H · Sur l'importance physiologique du nickel et du cobalt, Compt. rend Acad sc 185·321, 1927.
11 Bornstein, J: A technique for the assay of small quantities of insulin using alloxan-diabetic, hypophysectomized and adrenalectomised rats, Australian J Exper. B.ol. & M Sc 28.87, 1950
12 Bornstein, J, Reid, E, and Young, F. G: The hyperglycemic action of blood from animals treated with growth hormone, Nature, London 168 903, 1951
13. Bridge, E M The action of insulin on glycogen reserves, Bull Johns Hopkins Hosp 62 408, 1938
14 Bruch, E Recent progress in insulin physiology, Arch Int Med 86 427, 1950
15 Burger, M Die klinische Bedeutung der initialen Insulinhyperglykamie, Klin Wchnschr 9 104, 1930
16 Burger, M Die klinische Bedeutung des hyperglykamisierenden Prinzips im Pankreas, Klin Wchnschr 10.351, 1931
17 Burger, M Insulin und Glukagon in ihren Beziehungen zum Diabetes, Klin Wchnschr 16 361, 1937
17a Burger, M Insulin und Glukagon, Ztschr ges inn Med 2 311, 1947
18 Burger, M · Das Glukagon, Fortschr Diag u Therap 1 1, 1950
18a Burger, M., and Klotzbucher, E Der Nachweis zuckererregender Hormone (Insulin, Glukagon) im menschlichen Blute durch Transfusion, Ztschr ges inn Med 2 43, 1947
19 Burger, M, and Kramer, H Über den hepatischen Angriffspunkt des Insulins, Die primare paradoxe Insulinhyperglykamie, Ztschr ges exper Med 65 487, 1929.
20 Burger, M, and Kramer, H Primare Hyperglykamie und Glykogenverarmung der Leber als Folge intraportaler Insulininjektion nach Untersuchungen am Hund, Ztschr ges exper Med 67 441, 1929
21 Campbell, J, *et al* Diabetogenic effect of purified growth hormone, Endocrinology 46 273, 1950.

64. Foà, P. P; Weinstein, H R., and Smith, J A. Secretion of insulin and of a hyperglycemic substance studied by means of pancreatic-femoral cross-circulation experiments, Am. J. Physiol 157:197, 1949
65 Foà, P P, *et al.* Effects of the hyperglycemic-glycogenolytic factor (HGF), epinephrine and insulin in normal and depancreatized dogs, Proc. Soc Exper Biol & Med 80 635, 1952.
66 Foà, P. P, *et al.*, Secretion of the hyperglycemic-glycogenolytic factor in normal dogs, Am. J. Physiol 171 32, 1952
67. Foà, P. P, *et al*: Extraction of the hyperglycemic-glycogenolytic factor (HGF) of the pancreas with liquid ammonia, Science 117 82, 1953
68. Foà, P P, *et al.* Anterior pituitary growth hormone (STH) and pancreatic secretion of glucagon (HGF), Proc Soc Exper Biol & Med 83 758, 1953
69. Foà, P. P., *et al* Unpublished data
70. Gaede, K.: Neues vom Glukagon, Ztschr ges inn Med 7 364, 1952
71. Gaede, K; Ferner, H, and Kastrup, H. Über das zweite Kohlenhydratstoff-wechselhormon der Bauchspeicheldruse (Glukagon) und seine Herkunft aus den a-Zellensystem, Klin Wchnschr. 28:388, 1950.
72. Gaston, E A Total pancreatectomy, New England J Med 238 395, 1948.
73 Geiling, E M K, and DeLawder, A M · Studies on crystalline insulin. XI Does insulin cause an initial hyperglycemia? J Pharmacol Exper. Therap 39 369, 1930
74. Goldner, M G, and Clark, D E The insulin requirement of man after total pancreatectomy, J. Clin Endocrinol 4 194, 1944.
75 Gomori, G Observations with differential stains on human islets of Langerhans, Am. J Path 17 395, 1941
76 Ham, A. W, and Haist, R E · Histological study of trophic effects of diabetogenic anterior pituitary extracts and their relation to the pathogenesis of diabetes, Am J. Path 17 787, 1941
77. Heard, R D H, *et al* An α-cell hormone of the islets of Langerhans, J Biol. Chem 172 856, 1948
78 Hess, W. Zur histologischen Diabetesdiagnose an den Langerhansschen Inseln, Schweiz Ztschr Path u Bakt 9 46, 1946
79. Hill, D W, and Howitt, F O *Insulin, Its Production, Purification and Physiological Action* (London Hutchinson's Scientific and Technical publications, 1936)
80 Houssay, B A, and Foglia, V G La diabetes metahipofisaria, Rev Soc. argent biol 20 247, 1944, *see also* 4 859, 1928
81 Jensen, H F *Insulin Its Chemistry and Physiology* (New York Oxford University Press, 1938).
82 Kadota, I Studies on experimental diabetes mellitus as produced by organic reagents, J Lab & Clin Med, 35 568, 1950
83 Kadota, I, and Midorikawa, O Diabetogenic action of organic reagents· Destructive lesions of islets of Langerhans caused by sodium diethyldithiocarbamate and potassium ethylxanthate J Lab & Clin Med. 38 671, 1951
84 Kazal, L A, *et al* Hyperglycemic properties of crude pancreatic proteins, Proc Soc Exper Biol & Med 74 8, 1950
85 Kepinov, L. Système glycogénolytique hormonal Sur le *mécanisme de*

analysis of cobalt-hyperglycemia, Proc. Soc Exper Biol & Med. 84 383, 1953
43. Ellis, S; Anderson, H L, Jr., and Turtle, M A: A study of the mechanism of action of epinephrine on liver slices, J. Pharmacol & Exper Therap 106 383, 1952.
44 Elrick, H.: Pancreatic hyperglycemic factor and growth, Proc. Soc. Exper Biol. & Med 82.76, 1953.
45 Engel, F. L: The endocrine control of metabolism, Bull New York Acad. Med 29 175, 1953
46. Eser, S: The hyperglycemic and glycogenolytic factor of the pancreas, Bull. Fac. med. Istanbul 14.242, 1951 (abst in Chem. Abst. 46.2160g, 1952).
47 Esselborn, V. M., Davie, C D, and Hamblen, E C. Effect of the menarche on hepatomegaly in an adolescent girl with syndrome resembling glycogen disease, J Clin Endocrinol 10 339, 1950
48. Evans, G. The effect of insulin on cardiac and liver glycogen, Am J. Physiol 134 798, 1941.
49 Fasoli, A, *et al.* Serum lipoproteins in experimental diabetes In press
50 Ferner, H: *Beitrage zur Histobiologie der Langerhansschen Inseln des* Menschen mit besonderer Bei[illegible] hung *zum* Pankreasdiabetes, [illegible]
51 Ferner, H.· Studien uber die [illegible] speicheldruse und den Diabetes [illegible] 390, 1951
52 Ferner, H Der Diabetes mellitus als Relationsverschiebung der beiden inkretorischen Komponenten des Inselsystems, Materia Med Nordmark 4 3, 1952
53 Ferner, H Zur Bedeutung des Glukagons, Ärztliche Praxis 4 6, 1952
54 Ferner, H. *Das Inselsystems des Pankreas Entwicklung, Histologie und Pathophysiologie mit besonderer Berucksichtigung des Diabetes mellitus* (Stuttgart· Glory Thieme, 1952).
55 Fiessinger, N, *et al* L'équilibre glycémique du sang dans la perfusion hépatique à la fois portal et artérielle, Compt rend. Soc. biol. 122 32, 1936
56 Fiessinger, N, *et al* Influence de l'insuline sur la glycémie au cours de la perfusion du foie, Compt rend, Soc biol 124 952, 1937
57 Fineberg, S K, and Altschul, A The encephalopathy of hyperinsulinism, Ann Int Med 36 536, 1952
58 Fisher, N F Preparation of insulin, Am J Physiol 67 57, 1923
59 Foà, P P The meaning of the glucose tolerance curve, Chicago Med School Quart 10 5, 1949
60 Foà, P P *Progress in diabetes A review, Chicago Med School Quart.* 13 1, 1951
61 Foà, P P Glucagon The anti-insulin hormone of the pancreas, Chicago Med School Quart 14 145, 1953
62 Foà, P. P, and Weinstein, H R Metabolic effects of an insulin-free pancreatic extract, Am J Physiol 163 711, 1950
63 Foà, P P, and Weinstein, H R An insulin-free pancreatic extract capable of reducing the concentration of ketone bodies in the blood, Federation Proc 10 43, 1951

103 Meduna, L. J.: *Oneirophrenia* (Urbana, Ill.: University of Illinois Press, 1950)
104 Meythaler, F., and Brüning, A · Experimentelle Untersuchungen ueber den Ablauf des Kohlenhydratstoffwechsels in verschiedenen Gefassbereichen nach Darreichung von Glucose, Insulin, und Adrenalin, *Arch f. exper. Physiol u. Pharmakol.* 185·203, 1937
105 Mirsky, I. A, *et al* · The influence of pancreatectomy on the metabolic state of the alloxan-diabetic dog, *Endocrinology* 49.73, 1951
106 Morgan, M S, and Pilgrim, F J: Concentration of a hyperglycemic factor from urine of schizophrenics, *Proc Soc Exper. Biol & Med* 79.106, 1952.
107. Murlin, J. R, *et al.*: Aqueous extracts of the pancreas I Influence on the carbohydrate metabolism of depancreatized animals, J. Biol Chem 56 253, 1923.
108 Myers, J D, *et al* Endocrine influences on splanchnic carbohydrate balance in man, Proc Internat. Physiol Congress (19th), Montreal, 1953, p 638
109. Olsen, N. S, and Klein, J R. Hyperglycemia induced by certain insulin preparations, Proc Soc. Exper Biol & Med. 66 86, 1947.
110 Pincus, I. J A hyperglycemic factor extracted from the pancreas, Proc Am Diabetes A 9·39, 1949, *see also* J Clin Endocrinol 10 556, 1950
111. Pincus, I J.. Effect of hyperglycemic-glycogenolytic factor on blood sugar of patients with diabetes, Proc Soc. Exper Biol & Med 74 377, 1950
112. Pincus, I J: The hyperglycemic factor of the pancreas, Tr New York Acad Sc 14 319, 1952.
113 Pincus, I J, *et al* The effect of hyperglycemic-glycogenolytic factor on muscle glycogen, Proc Internat Physiol Congress (19th), Montreal, 1953, p 660
114 Rathery, F, and Levina, L Influence des sels de nickel et de cobalt sur quelques diabétiques, Compt rend Acad sc 183.326, 1926
115 Ricketts, H T, Brunschwig, A, and Knowlton, K. Effects of total pancreatectomy in a patient with diabetes, Am. J Med 1 229, 1946
116 Rodriguez-Candela, J L.. Clinica de la diabetes por aloxana, Rev clin españ 19 393, 1945.
117 Rodriguez-Candela, J L The hyperglycemic-glycogenolytic factor produced by the pancreas, J Clin. Endocrinol 12·245, 1952
118 Rodriguez-Candela, J L, and Cristensen-Konig, W Accion antiinsulínica del páncreas, Trab Inst nac ciemas méd (Madrid) 18 31, 1950
119 Rodriguez-Candela, J L, *et al.* Influencia del extracto de páncreas aloxánico sobre la acción de la insulina en el consumo de glucosa por el diafragma aislado, Arch med exper 15 91, 1952
120 Rowlinson, H R, and Lesford, J. M An *in vivo* test for the hyperglycemic-glycogenolytic factor in insulin, J Pharm & Pharmacol 3 887, 1951.
121 Saka, M O. Hyperglycemic-glycogenolytic factor in diabetic man and alloxan-diabetic animals, Am J Physiol. 171.401, 1952
122 Scott, D A Crystalline insulin, Biochem J 28 1592, 1934
123 Shipley, R A, and Humel, E J, Jr Carbohydrate and acetone body metabolism of liver slices and effect of insulin, Am J Physiol. 144 51, 1945

l'action glycogénolitique de l'adrénaline et le rôle de l'hormone hypophysaire dans ce mécanisme, Arch Internat. physiol 46 265, 1938
86 Kibler, R. F., and Myers, J D: Responsiveness of the pancreatic hyperglycemic-glycogenolytic factor (HGF) as a test of liver function, Nat'l Meet Am. Fed. Clin Res, Atlantic City, N. J, May, 1953.
87. Kimball, C. P., and Murlin, J. R: Aqueous extracts of pancreas III Some precipitation reactions of insulin, J. Biol. Chem. 58·337, 1923
88 Kinash, B, *et al*. Effects of anterior pituitary extracts and of growth hormone preparations on the islets of Langerhans and the pancreas, Diabetes 2:112, 1953
89 Kirtley, W R., Waife, S O, and Peck, F. B. Effect of glucagon in stable and unstable diabetic patients, Proc. Soc. Exper. Biol. & Med 83 387, 1953
90. Labbé, M, Roubeau, H, and Nepreux, F.: Influence du nickel et du cobalt sur l'action hypoglycémiante de l'insuline chez le lapin, Compt. rend Acad. sc 185 1532, 1927
91. Labbé, M; Roubeau, H, and Nepreux, F.: Action des sels de nickel et de cobalt sur le pouvoir hypoglycémiant de l'insuline chez le diabètique, Compt rend Acad. sc 186 181, 1928
92 Larsson, J· Über den vermutlichen Entstehungsort des Glucagon in der Magen-Darmschleimhaut, Deutsche Ztschr. Verdauungskr. 11.294, 1951.
93. Lazarus, S S, Volk, B W., and Goldner, M G. The physiologic consequences of selective destruction of the alpha cells of the pancreatic islets, 35th Meeting Endocrine Soc, New York, 1953
94. Le Goff, J. M· Cobalt as vaso-dilator, J Pharmacol & Exper Therap. 38.1, 1930
95 Looney, J M, and Cameron, D E Effect of prolonged insulin therapy on glucose tolerance in schizophrenic patients, Proc. Soc. Exper. Biol & Med. 37 253, 1937
96 Loube, S D, Campbell, E D, and Mirsky, I A Administration of hyperglycemic-glycogenolytic factor (HGF) of the pancreas to non-anesthetized and anesthetized subjects, Proc Soc. Exper Biol & Med 75 161, 1950
97 Lundsgaard, E., Nielsen, N A, and Orskov, S L On the possibility of demonstrating an effect of insulin on isolated mammalian liver, Skandinav Arch Physiol 81 11, 1939
98 McQuarrie, I, Zimmermann, B, and Wright, W S Deficiency of alpha cells of pancreas as the possible etiological factor in familial hypoglycemosis, Federation Proc 9 337, 1950
99 Malandra, B, and Mosca, L. Il fattore iperglicemizzante pancreatico nei teleostei, Rassegna fisiopat clin e terap 24 43, 1952
100 Marks, H P, and Young, F G Observations on the metabolism of dogs made permanently diabetic by treatment with anterior pituitary extract, J Endocrinol 1 470, 1939
101 Maya, F Personal communication
102 Mayer, J.. Rôle d'une hormone sécrétée par les cellules des ilots de Langerhans ("*nésocrine*") dans l'étiologie du syndrome "obésité-hyperglycémie héréditaire," Compt, rend Acad sc 236 1604, 1953

142 Vuylsteke, C, and de Duve, C: The glucagon content of avian pancreas, Proc. Internat Physiol Congress (19th), Montreal, 1953, p 860.
143 Vuylsteke, C. A; Cornelis, G, and de Duve, C: Influence du traitement au cobalt sur le contenu en facteur H-G du pancréas de Cobaye, Arch internat physiol. 60.128, 1952
144. Vuylsteke, C. A., de Duve, C., and Nys, A: Étude sur le facteur H-G du pancréas, Arch internat. physiol 57·445, 1950
145. Waters, E. T. Review of studies on blood sugar, Bull New York Acad Med. 25 32, 1949.
146 de Waele, H: Sur une hormone pancréatique à action opposée à celle de l'insuline, Arch internat. physiol 53.209, 1948, 57:133, 1949.
147. Weisberg, H. F., and Kaplan, C M.· Relationship of cobalt chloride to hyperglycemic-glycogenolytic factor (HGF), Federation Proc. 12 151, 1953
148. Weisberg, H F, and Kaplan, C. M.· Effect of cobalt on tissue glycogen, Proc. Internat. Physiol Congress (19th), Montreal, 1953, p. 876.
149. Weisberg, H F., and Schaefer, G L.· Possible relationship between carcinoid (argentaffin) tumors and the hyperglycemic-glycogenolytic factor (HGF), Am J. Clin Path. 22.1169, 1952.
150. Weisberg, H. F., *et al.*· Effects of the hyperglycemic-glycogenolytic factor (HGF) found in insulin preparations, Am J Physiol 159.98, 1949
151. Werch, S C, and Altshuler, S S A study of the blood sugar raising substance in the urine of diabetic and non-diabetic patients, Am. J Physiol 118 659, 1937.
152 Young, F G· Interrelationship of pituitary and pancreas, Proc Internat Physiol. Congress (19th), Montreal, 1953, p 18
153. Zanoboni, A *Il Cobalto nel Diabete Mellito* (Doctoral Thesis, University of Milan, 1953).
154 Zunz, E, and La Barre, J Sur l'augmentation de la teneur en insuline du sang veneux pancréatique après l'hyperglycémie provoquée par injection de glucose, Compt rend Soc biol 96 421, 1927
154a Zunz, E, and La Barre, J Sur les causes de l'augmentation de la teneur en insuline de sang veineux pancréatique lors de l'hyperglycémie provoquée par injection de dextrose, Compt rend Soc. biol. 96 708, 1927
154b Zunz, E, and La Barre, J L'hypoglycémie insulinique dominne-t-elle la production d'insuline par le pancréas? Compt rend Soc biol 96 1045, 1927.

Addendum

155 Coulson, R A., and Hernandez, T. Glucose in crocodilia, Endocrinology, 53 311, 1953
156 Ellis, S, Anderson, H L, Jr, and Collins, M C Pharmacologic differentiation between epinephrine and HGF-hyperglycemias Application in analysis of cobalt-hyperglycemia, Proc Soc Exper Biol & Med 84 383, 1953.
157. Fabrykant, M. The alpha cells of pancreatic islets and the pathogenesis of diabetes mellitus, Am J Digest Dis, 20 275, 1953
158 Geschwind, I I, and Staub, A Lack of growth-promoting potency and of toxicity of glucagon (hyperglycemic-glycogenolytic factor) in hypophysectomized rats, Proc Soc Exper Biol & Med. 84 244, 1953

124 Somogyi, M: Studies of arteriovenous differences in blood sugar, J. Biol. Chem 174 189, 597, 1948, 179 217, 1289, 1949, 186 513, 1950
125. Somogyi, M: Effect of insulin injections repeated at brief intervals, Endocrinology 47:436, 1950
126 Sonenberg, M, *et al* Radioactive growth hormone preparations, J Clin Endocrinol 12 938, 1952.
127. Sopp, J. W.. Über das Glukagon, die hyperglykamisierende Substanz des Pankreas, Ztschr ges exper. Med. 96 817, 1935.
128. Staub, A.; Sinn, L, and Behrens, O K· Purification and crystallization of hyperglycemic-glycogenolytic factor (HGF), Science 117.628, 1953.
129. Staub, A; Sinn, L, and Behrens, O K.: Crystalline glucagon, Proc. Internat Physiol Congress (19th), Montreal, 1953, p 797.
130. Stewart, R D, and Roitman, E. Effect of pancreatic extracts on ketone body production of rat liver, Endocrinology 53 192, 1953.
131 Sutherland, E W.. The effect of the hyperglycemic factor and epinephrine on enzyme systems of liver and muscle, Ann. New York Acad. Sc 54 693, 1951
131a Sutherland, E W.. The effect of the hyperglycemic factor of the pancreas and of epinephrine on glycogenolysis, Recent Progress in Hormone Res. 5·441, 1950
132 Sutherland, E W, and Cori, C F· Influence of insulin preparations on glycogenolysis in liver slices, J Biol. Chem 172·737, 1948.
133 Sutherland, E. W, and Cori, C F· Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase, J Biol. Chem 188 531, 1951.
134 Sutherland, E W, and de Duve, C. Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas, J Biol Chem 175 663, 1948
135 Sutherland, E W, *et al* Purification of the hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa, J Biol Chem 180 825, 1949
136 Thorogood, E, and Zimmermann, B The effects of pancreatectomy on glycosuria and ketosis in dogs made diabetic by alloxan, Endocrinology 37 191, 1945
137. Tyberghein, J Action du glucagon sur le métabolism des protéines, Arch. intern physiol 61 104, 1953
138 Van Abeele, F R, and Campbell, E D Action of crystalline trypsin on insulin and the hyperglycemic factor, Federation Proc 10 263, 1951
139 van Campenhout, E, and Cornelis, G Destruction expérimentale des cellules alpha des îlots endocrines du pancréas chez le cobaye, Bull Acad. roy méd Belgique 16 382, 1951
139a van Campenhout, E, and Cornelis, G Destruction expérimentale des cellules alpha des îlots endocrines du pancréas, Compt rend Soc biol 145 933, 1951
140 Volk, B W Goldner, M G, and Lazarus, S S The selective destruction of the alpha cells by cobaltous chloride and its physiologic implications, Am J Path 29 580, 1953
141 Volk, B W, Lazarus, S S, and Goldner, M G Alpha cell damage and blood sugar changes in rabbits after administration of cobalt, Proc Soc Exper. Biol & Med 82 406, 1953

Diagnosis of Cancer of Internal Organs by Papanicolaou Technic

RUTH M. GRAHAM

Vincent Memorial Laboratory in Vincent Memorial Hospital, and the Gynecologic Service of Massachusetts General Hospital, Boston

THE DIAGNOSIS of cancer by single cells has been widely used for scarcely a decade Occasional observers, such as MacCarty (48), over the years have doubted the necessity of a histologic section to recognize the malignant character of cells, but they were largely ignored until 1942 It was only after Papanicolaou and Traut (61) reported impressive accuracy of diagnosis of uterine cancer by examination of the vaginal smear that great interest in single malignant cells developed The literature since then has become voluminous Article after article has appeared confirming Papanicolaou's original investigation, and interest has broadened to include the search for and study of malignant cells in sputum, gastric juice, colonic washings, breast secretion, and urine, to mention only the major sites investigated It is virtually impossible and unnecessarily confusing to attempt in a review such as this to cover the entire literature on exfoliative cytology It is more pertinent to discuss the field from the point of view of its uses, advantages, and limitations.

The cytologic diagnosis of cancer is possible because cells are constantly being shed from the epithelial surfaces of the body. Vaginal smears contain cells desquamated from the surface of the vagina, cervix, and endometrium Sputum contains cells shed from the bronchi and upper respiratory tract If there is a malignant growth in an epithelium, cancer cells at the surface will desquamate just as the benign cells do. If the cells are then properly collected, fixed, and stained,

159. Ingle, D. J., Nezamis, J E., and Humphrey, L M. Absence of hyperglycemic effect of glucagon in the eviscerated rat, Proc. Soc Exper. Biol & Med 84.232, 1953
160 Kayser, C. L'hibernation des mammifères, Proc. Internat Physiol Congress (19th), Montreal, 1953, p 128
161. Malandra, B, Fior, R., and Galansino, G.: Effetto dell' ormone somatotropo ipefisario sul diabete allossanico del ratto, Boll Soc. ital pat. 3 1, 1953
162. Mayer, J.; Andrus, S. B, and Silides, D. J.: Effect of diethyldithiocarbamate and other agents on mice with obese-hyperglycemic syndrome, Endocrinology 53 572, 1953.
163. Pincus, I J, and Rutman, J. Z Glucagon, the hyperglycemic agent in pancreatic extracts, A M. A Arch Int. Med 92,666, 1953
164. Volk, B. W.; Lazarus, S S., and Goldner, M. G.: Alpha cells of pancreas Morphologic and physiologic considerations, A. M A. Arch Int. Med 93 87, 1954
165 Cavallero, C, *et al.* Osservazioni preliminari sugli effetti morfologici e biochimici da glucagone nel ratto, Boll Soc ital pat. 3 3, 1953
166 Cavallero, C., and Mosca, L: Mitotic activity in the pancreatic islets of the rat under pituitary growth hormone and adrenocorticotropic hormone treatment, J Path. & Bact 66 147, 1953
167. Cavallero, C Études sur le facteur hyperglycémiant du pancréas (glucagon), Rev canad biol 12,189, 1953
168 Kracht, J.. Wirkung von Wachstumshormon auf die Langerhansschen Inseln des Rattenpankreas, Naturwissenschaften 40 607, 1953
169 MacGrath, W B., Jr, and Snedecor, J G The effect of insulin on the glucagon content of the pancreas, Diabetes 2 443, 1953
170 Haugaard, E S, and Haugaard, N The effect of hyperglycemic-glycogenolytic factor on fat metabolism of liver, J Biol Chem 206 641, 1954

but is in large clumps and strands. The condensation of the chromatin in large particles often leaves empty spaces. The chromatin is condensed at the border of the nucleus, producing a sharp, well-defined nuclear rim. This change in chromatin distribution may be accompanied by an increase in the amount of chromatin. However, one must bear in mind that the amount of chromatin present is by no means a reliable index of malignancy. The cell that is big and black is not necessarily malignant, nor is the pale, lightly staining nucleus benign

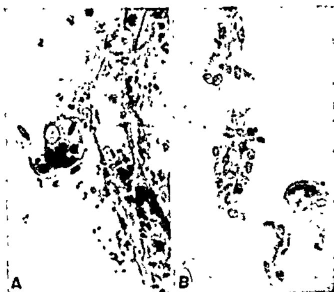


FIG 2—*A*, cells from a squamous carcinoma of the lung *B*, normal ciliated columnar cells from bronchial epithelium as seen in sputum

The nuclei of shed cells may undergo pyknotic changes, with condensation of chromatin, or chromatolysis, with solution of the nucleoprotein. These occur in benign and in malignant cells.

As with the normal cell, the origin of a malignant cell is indicated by the cytoplasm Granted, there are some malignant cells in which this is impossible, since if no definite cytoplasm is present the cancer cell must be considered undifferentiated However, if cytoplasm is present, the type of tumor may be stated with reasonable accuracy If cytoplasm is present but the shape is distinctly abnormal, as in the fiber cell in Figure 1, we can assume that the cell has desquamated from a squamous carcinoma If the malignant nucleus is eccentrically placed

characteristic differences between malignant and benign cells may be seen microscopically.

The cytologic diagnosis of cancer invariably depends on the appearance of the nucleus. We are aware of no morphologic character- [illegible]

divided particles, or, as one student expressed it, the nuclei have "a salt

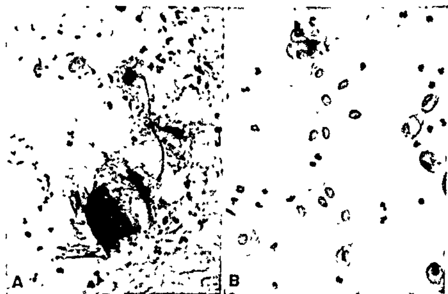


FIG 1—A, malignant fiber cells from squamous carcinoma of the cervix B, normal basal cells as seen in the vaginal smear

and pepper" appearance. This applies to well-preserved cells from many different sites. the squamous cells of the lower genital tract, the columnar cells from the endometrium, bronchi, or gastric mucosa, and the transitional cells from the urinary tract The decision whether a cell is benign or not depends on the nuclear structure. What type of normal cell it is depends on the cytoplasm An example of this is the normal columnar cells from the respiratory tract with definite cilia, in contrast to those of the gastrointestinal tract in which no cilia are present.

In contrast, the appearance of the nuclear material in the malignant cells is irregular It is no longer finely granular and evenly distributed

in the cell and the cytoplasm finely vacuolated (Fig. 3), we can be quite certain that we are dealing with an adenocarcinoma. In Figures 1–4, benign cells with their malignant counterparts are shown, to illustrate the characteristics of both. It is particularly helpful to identify the type of tumor cell in the unsuspected case. It will suggest to the clinician, for example, whether the tumor is in the cervix or in the fundus of the uterus.

CANCER OF FEMALE GENITAL TRACT

The importance of the technic by which the specimen is obtained

men is relatively simple, it must be rigidly adhered to.

Several different types of specimens may be obtained for the diagnosis of uterine cancer.

1. *Vaginal secretion.*—This accumulates in the posterior fornices and can be aspirated. It is collected by means of a blunt glass pipet with a 1 to 2 mm. capillary opening It is necessary to have a small opening in the tube because the vaginal mucus is so tenacious that it adheres to the walls of the glass. If the opening is large, it is difficult to blow the secretion onto the glass slide, for it sticks to the side of the pipet With a smaller opening, the material is evacuated more readily.

The secretion is placed on a marked glass slide. We have preferred to distribute the material by spreading it on the slide with the tip of the pipet In such smears there are sufficiently thin areas which can easily be read. Others, for example Reagen and Schmidt (68), have preferred to overlay one slide with another, then pull them apart, as for blood smears. Whatever method is used, it must be done quickly, and the slide placed in the fixative of ether and alcohol immediately, before it has a chance to dry. This method is based on the wet fixation of the cells and not on air-dried smears, as in hematology

2 *Cervical scraping.*—This type of smear, introduced by Ayre (3), is preferred by some cytologists. The surface of the cervix is scraped and the cells obtained are placed on a slide, then treated as described for the vaginal smear The method has the advantage that more atypical cells are found, decreasing the time required to screen the slide. It has been stated (20) that scraping the cervix yields more accurate results in the detection of cervical cancer than can be obtained by

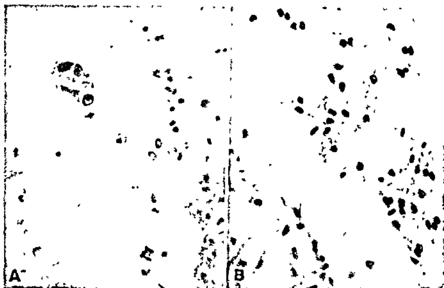


FIG 3—*A*, cells from adenocarcinoma of the stomach *B*, normal columnar cells from gastric mucosa. Note that they are not nearly as well preserved as those from sputum.

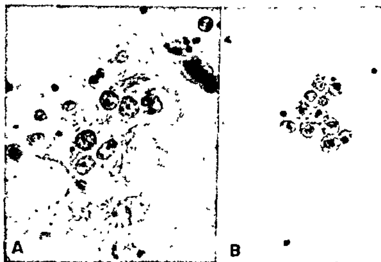


FIG 4—*A*, undifferentiated cancer cells from a carcinoma of the bladder. *B*, normal cells from urine

what from laboratory to laboratory, and depends to a great extent on the experience of the cytologist. Table 1 gives figures for the diagnosis of cancer of the cervix from several clinics. Carcinoma of the cervix is diagnosed in about 90 per cent of the cases in most laboratories.

The obvious advantage and usefulness of the method is in the detection of early cervical carcinoma. It is particularly gratifying that by this method the early cases of cervical cancer without clear-cut symptoms or signs of malignancy can be discovered. Cytologic examination is more likely to miss the far-advanced cervical tumor than

TABLE 1—CERVICAL AND ENDOMETRIAL CANCER DIAGNOSED BY CYTOLOGIC SMEAR METHOD

REFERENCE	CASES	CERVICAL CANCERS		ENDOMETRIAL CANCERS		% FALSE POSITIVE*
		No	% Diagnosed	No.	% Diagnosed	
Papanicolaou and Traut (61)	3,014	127	96.9	53	86.8	0.0
Jones *et al.* (43)	427	48	100.0	40	82.5	9.9
Burns *et al* (7)	6,437	54	85.2	20	50.0	24.3
Gusberg and Graham (33)	1,000	49	87.8	22	77.3	16.0
Reichter *et al* (69)	3 500	67	94.1	18	72.1	18.2
Cuyler *et al.* (9)	15,000	447	91.5	44	65.9	10.8
Reagen and Schmidt (68)	1,000	53	98.2	34	88.3	3.7
Sheffey *et al* (75)	500	57	70.1	6	66.6	13.7
Graham (29)	18,302	641	89.6	2 6	71.2	9.8
Gates and Warren (24)	3,645	238 (uterine)	86.6			11.0
Ayre (4)	2,320	226 (uterine)	95.2			10.6

* Calculated on cases called positive cytologically, both cervical and endometrial

the small in situ lesion, for the method depends on having well-preserved cells shed from the surface of the cancer. But in the far-advanced neoplasm the surface is often necrotic and the specimen contains only necrotic material.

The category of early or symptom-free cancer includes early invasive carcinoma and in situ carcinoma of the cervix. Many of the cases of unsuspected cancer will fall in the in situ group In our laboratory, of 81 unsuspected cases of uterine cancer, 43, or 53 per cent, were classified as in situ histologically The accuracy of the cytologic examination in detecting carcinoma in situ is somewhat slighter than for invasive squamous carcinoma. As already mentioned, carcinoma in situ of the cervix was diagnosed in our laboratory in 86 per cent of the

vaginal smear. However, the differences in large series are not significant. Foote and Li (20) reported on 18 cases of carcinoma in situ in which both cervical scrapings and vaginal smears were examined. The cervical scrapings were positive in 14, an accuracy of 78 per cent, vaginal smears were positive in 9, or 50 per cent. In 86 cases of carcinoma in situ seen at the Vincent Laboratory (29), the first vaginal smear was positive in 72, a percentage accuracy of 86. These figures are not significantly different in the recognition of cancer cells in carcinoma in situ.

One disadvantage of the cervical scraping method is that a dry speculum must be inserted, which usually requires the services of a physician. A vaginal smear may be taken by a technician on a patient in bed A further disadvantage is that great care must be taken to scrape the squamocolumnar junction of the cervix, where cervical tumors are most apt to develop, and not merely the exocervix. The vaginal smear contains cells not only from the cervix but from the vagina, endometrium, and occasionally tube and ovary. Therefore, it actually gives a better sample of cells from all portions of the female genital tract. Some clinics have recognized this problem and have taken both a vaginal smear and a cervical scraping At the Vincent Laboratory, it has been the policy to take a single vaginal smear and to reserve the *cervical scraping method for difficult diagnostic problems.* Direct smears from the endocervix and endometrium may be taken with a sterile cannula and the aspirate prepared as for vaginal smears

The smears must remain in the fixative for at least an hour to have the cells adhere firmly to the slide They may stay in the fixative indefinitely. If the slides are to be mailed to a cytology laboratory, they may be taken out of the fixative after 24 hours, dried, wrapped in paper, and sent

The majority of cytologic smears are stained by the Papanicolaou method (57). This is an excellent trichrome stain The nuclear stain is a Harris hematoxylin Obviously, whatever the stain used, the nuclear one must be a good one, since so much depends on the appearance of the nuclei Such stains as Giemsa's and Wright's are not particularly good for this type of specimen

Since Papanicolaou's original article appeared, his work has been amply confirmed Confirmation has come from large teaching centers and from small general hospitals There can be no doubt that cancer of the uterus can be diagnosed with an accuracy comparable to that of a single biopsy specimen. The accuracy of the method varies some-

genital tract was less than 1 per cent. In those with symptoms, it was 10 per cent. Fremont-Smith and Graham (23), in screening new women patients consulting a general practitioner, discovered 10 cases in 714, or an incidence of 1.4 per cent, even though only 13 per cent had symptoms referable to the genital tract. It may be that a routine vaginal smear should be done on the women who seek medical help for any specific symptom, rather than on those who come for a general check-up.

The method continues to be valuable after the diagnosis has been made. If the patient is treated by irradiation, the response of the tumor

TABLE 2.—CERVICAL CANCER FOUND BY CYTOLOGIC SMEAR METHOD IN VARIOUS CANCER DETECTION CENTERS

Reference	No. of Cases Examined	No. of Unsuspected Cases	Incidence, %
Reagan and Schmidt (68)	1,000	8	0 8
Skapier (76)	10,000	30	0 3
Gusberg and Graham (33)	419	15	3 5
Haynes (34)	6,816	36	0 5
Burns *et al* (7)	6,437	22	0 3
Peters and Madden (65)	200	3	1 5
Graham (29)	18,302	81	0 4
Fremont-Smith and Graham (23)	714	10	1 4
Kaufman and Fiege (44)	1,712	23	1 3
Nieburgs and Pund (53)	10,000	105	1 0
Anderson *et al* (2)	1,000	11	1 1
Erickson *et al* (16)	20,000	163	0 8

to treatment can be gaged with fair accuracy by an examination of the vaginal smear (27). The benign squamous epithelial cells show characteristic changes during radiotherapy. the cytoplasm becomes vacuolated, the cells increase in size, multiple nuclei are present, and the nuclei become irregular. If more than 75 per cent of these cells show the changes, the response is classified as good, if less than 60 per cent, the response is considered to be poor with respect to prognosis. A series of 125 patients was studied in this way at the time of their initial treatment and followed for 5 years (28). The response in 62 cases was classified as good, 36 of these (59 per cent) survived 5 years In 63 cases the response was considered to be poor, only 2 (3 per cent) survived 5 years. Nielsen (54) has confirmed this method of prognosis by vaginal smear.

cases. Gusberg and Graham (33) reported the diagnosis of 12 of 15 carcinomas in situ—an 80 per cent accuracy. Achenbach *et al.* (1) obtained a positive vaginal smear in 70 per cent of 60 cases of carcinoma in situ.

The number of in situ carcinomas of the cervix reported in recent years has increased markedly; in great measure, this is due to the fact that routine smears have been taken on increasing numbers of asymptomatic women.

It is pertinent to recall that the diagnosis of an in situ lesion is a histologic diagnosis, not a cytologic one As the descriptive name of this lesion indicates, it depends entirely on whether the tumor is contained within the epithelium. In about 50 per cent of the cases, the smears from patients with in situ carcinoma contain extremely well-differentiated malignant cells. However, in the remaining 50 per cent of carcinomas still histologically in situ, the smears have all the characteristics of invasive carcinoma. Biopsy must then be done to establish the type of lesion present, because on the basis of single cells alone it is impossible to know with certainty whether or not the tumor is invading underlying tissue. Cuyler (10) reported on 112 carcinomas in situ in which an attempt was made to decide whether or not the tumor was invasive or in situ by means of cytologic criteria; 25 of the cases were classified as invasive (22 3 per cent), 66 (58 9 per cent) as in situ, and 21 (18.9 per cent) were called negative. His report is recommended as a comprehensive review of the feasibility of recognizing in situ carcinoma cytologically

Because the cytologic method can detect very early cancers of the cervix, it has been used in screening programs, and is used routinely in the cancer detection centers. In recent years there have been an increasing number of reports from such centers Table 2 shows the number of cases found per 1,000 examinations in several centers The figures are fairly consistent The usual yield is between 3 and 15 per 1,000 in the examination of well women, with the exception of Gusberg and Graham's figures which show a rate of 35 per thousand. This figure has not been attained by any other clinic. It may be, as Lombard, *et al* (47) pointed out, more pertinent to screen those patients with symptoms In a study done by the Massachusetts State Department of Health, the women studied were divided into two groups—those with no signs or symptoms of malignancy, and those with some symptom suggesting malignancy, such as abnormal bleeding. The incidence of uterine cancer among women with no complaints referable to the

logically, between a well-differentiated endometrial carcinoma and a hyperplastic endometrium. (2) *If the cells are collected in a cervical or vaginal aspirate, they may be degenerate, probably because some time elapses before the cells arrive at the cervix or vagina.* Papanicolaou and Marchetti (60) recognized this difficulty and largely overcame it by using a sterile cannula for aspirating the endometrial cavity, with a resultant increased accuracy of diagnosis. The technic may be used in a doctor's office.

If an unsuspected case of uterine cancer is considered to be one in which there are no symptoms referable to malignancy and the physical examination is negative, only a few such cases have been reported. In the majority of instances, abnormal bleeding occurs when the tumor *is still fairly small. At our laboratory, only 13 (6 per cent) of 206 patients with histologically proved endometrial cancers had not exhibited* suspicious symptoms or signs. Though this is lower than the percentage of cervical cancer detected, it deserves consideration. Direct aspiration of the endometrial cavity should result in a significant increase in the number of early cancers of the fundus that are discovered

Other malignant tumors of the female genital tract may be diagnosed by the cytologic method As might be expected, the accuracy of diagnosis *in vaginal cancer, either primary or recurrent, is excellent. Occasionally cancer cells have been found in the cervical and vaginal* secretions from both tubal and ovarian carcinoma without involvement of the endometrium (21, 49) Cancer cells may sometimes be found in the vaginal secretion from a tumor which is not in the genital tract Parsons and Taymore (63) report a case in which peritoneal seedings of a carcinoma of the breast were responsible for repeated positive vaginal smears. Malignant cells may occur if there is a fistulous tract from a bowel carcinoma to any portion of the female genital system Malignant cells may be found as contaminants in the vaginal smear of women with bladder carcinoma without any fistula between bladder and vagina These examples are given to suggest to the clinician that if repeated positive reports are returned from the laboratory and no tumor can be found in the genital tract, other systems should be investigated

Thus far, we have considered the accuracy of the cytologic method in diagnosing uterine cancer The discussion has been an attempt to answer the question frequently asked "If my patient has cancer, how often may I expect a positive report?" There is a certain definite error,

Recent studies have indicated that an index of the radiosensitivity of the tumor can be obtained by examining the normal cells of the vaginal smear before any treatment has been given in cases of invasive *cervical cancer (30). The basal cells of the squamous epithelium have* a dense, bluish-gray cytoplasm and fine vacuolization. This change in the basal cells is called the sensitization response (SR). If more than 10 per [illegible] the pa [illegible] view, [illegible] sidered poor. In 100 unselected cases of invasive cervical cancer treated by radiotherapy, the amount of SR was correlated with 5 year survivals There were 35 with significant SR, the 5 year survival rate in this group was 66 per cent. In the 65 patients without significant SR, the 5 year survival rate was 18 per cent. Thus, by examination of the control vaginal smear, some indication of how the tumor will react to *radiation may be obtained.*

Even after treatment has been concluded and the patient is being followed for possibility of recurrence, the role of the vaginal smear is still important It is extremely useful in the follow-up clinic. The accuracy of detection of local recurrence by means of the vaginal smear is 98 per cent (29) In no other single group of cases is the accuracy so high These are the cases in which use of the cytologic method is essential It is difficult to ascertain by clinical methods whether, after irradiation, an early local recurrence has developed. Additional information is required, and this can be supplied by the cytology laboratory It is not only useful in the postirradiation cases, but in the postoperative ones as well When an adequate vaginal cuff is removed, as in radical hysterectomy, evaluation of the remaining part of the vagina is difficult The usefulness of the method in unsuspected postoperative cancer recurrence has been reported by Graham and Meigs (26)

The discussion here has been limited to the usefulness of the cytologic method in the diagnosis and treatment of cancer of the cervix. While the method is particularly valuable in cervical cancer, it is also of value in *endometrial cancer,* although the accuracy of diagnosis is not as great From Table 1 it can be seen that in most clinics the accuracy is about 20 per cent less than that for cervical carcinoma. There are two reasons for this error: (*1*) The cellular differentiation of adenocarcinoma in single cells is more difficult than for those of squamous carcinoma, just as it may be more difficult to decide, histo-

logically, between a well-differentiated endometrial carcinoma and a hyperplastic endometrium. (2) If the cells are collected in a cervical or vaginal aspirate, they may be degenerate, probably because some time elapses before the cells arrive at the cervix or vagina. Papanicolaou and Marchetti (60) recognized this difficulty and largely overcame it by using a sterile cannula for aspirating the endometrial cavity, with a resultant increased accuracy of diagnosis. The technic may be used in a doctor's office.

If an unsuspected case of uterine cancer is considered to be one in which there are no symptoms referable to malignancy and the physical examination is negative, only a few such cases have been reported. In the majority of instances, abnormal bleeding occurs when the tumor is still fairly small. At our laboratory, only 13 (6 per cent) of 206 patients with histologically proved endometrial cancers had not exhibited suspicious symptoms or signs. Though this is lower than the percentage of cervical cancer detected, it deserves consideration. Direct aspiration of the endometrial cavity should result in a significant increase in the number of early cancers of the fundus that are discovered.

Other malignant tumors of the female genital tract may be diagnosed by the cytologic method. As might be expected, the accuracy of diagnosis in vaginal cancer, either primary or recurrent, is excellent. Occasionally cancer cells have been found in the cervical and vaginal secretions from both tubal and ovarian carcinoma without involvement of the endometrium (21, 49) Cancer cells may sometimes be found in the vaginal secretion from a tumor which is not in the genital tract. Parsons and Taymore (63) report a case in which peritoneal seedings of a carcinoma of the breast were responsible for repeated positive vaginal smears. Malignant cells may occur if there is a fistulous tract from a bowel carcinoma to any portion of the female genital system Malignant cells may be found as contaminants in the vaginal smear of women with bladder carcinoma without any fistula between bladder and vagina. These examples are given to suggest to the clinician that if repeated positive reports are returned from the laboratory and no tumor can be found in the genital tract, other systems should be investigated

Thus far, we have considered the accuracy of the cytologic method in diagnosing uterine cancer The discussion has been an attempt to answer the question frequently asked "If my patient has cancer, how often may I expect a positive report?" There is a certain definite error,

in cervical cancer a 10 per cent error, in fundal cancer a 20 per cent error. We must also answer a second question: "If I receive a positive report, how often will my patient be proved to have cancer histologically?" This is a more important question than the problem of the accuracy of negative reports. The negative cases run usually in the thousands, the number of actual cases of cancer called negative in the tens, so that the percentage of accuracy is above 99. This means very little in the individual case, however. What the clinician would like to know is with what frequency a false positive report is returned. How often will he receive a positive report which on further investigation is not confirmed? It is disturbing both to the patient and to the doctor to have a serious false alarm.

Here it is that everything depends on the experience and the conservatism of the cytologist (29). Any cytologist can almost always avoid all false positive reports by being extremely conservative and by diagnosing cancer only when he finds cancer cells about which there can be no doubt whatever. But when the cytologist's requirements are too severe, his errors in diagnosing cancer begin to increase This, too, is unfortunate, for although every case of cancer diagnosed as such will prove to be cancer, an increased number of cancers, perhaps early curable ones, will be missed. Thus the cytologist must attempt to strike the happy medium, he must diagnose the greatest possible number of cancers and make as few false positive diagnoses as possible. Table 1 shows the number of such reports by several investigators. As may be readily seen, there is a wider range of inaccuracy here than on the other side of the picture—the false positive. However, the method is obviously specific and accurate enough to warrant further study of the patient so as to find the source of the cancer cells

It is worth emphasizing that no definitive treatment should be undertaken on the basis of a positive cytology report. The site of the tumor cannot always be accurately determined, nor is it possible to determine from single desquamated cells whether the early lesion is still in situ Last, but not least, a certain percentage of the reports will be false positives.

The diagnosis of cancer by means of exfoliated cells was not intended as a substitute method for biopsy, but as a complementary one The first cytologic report and the first biopsy report were compared in a series of 183 primary cases of cervical cancer studied at our laboratory (31) The first biopsy was positive in 90 per cent of the cases, the first cytology examination in 91 per cent. There was no significant

difference, therefore. However, when the first biopsy report and the first cytology report were considered together, either one or the other was positive in 98.6 per cent of the cases. The diagnosis of cancer by examination of desquamated cells is thus an additional important method for detecting early cancer, but is not a substitute for existing, well-established technics.

PULMONARY CANCER

Perhaps the most important diagnostic use of the cytologic method is in the examination of sputum and bronchial secretions. The lung is not nearly as accessible to biopsy procedures as the female genital tract. Additional microscopic methods of diagnosis are essential and cytology has, partially at least, filled this need.

There have been sporadic flares of interest in the diagnosis of pulmonary cancer by examination of the sputum for many years. Dudgeon and Wrigley (13) in 1935 reported a series of cases, with an accuracy of diagnosis of 68 per cent. In 1938, Gowar (25) reported comparable figures. However, there were few confirmations of their work, and it was not until 1944, when Wandell (80) reported on a series of 100 with an accuracy of diagnosis of 84 per cent that interest was renewed By that time, Papanicolaou's observations on the cytologic diagnosis of uterine cancer had been confirmed and there was increasing interest in the cytologic method. Investigators turned to the examination of the sputum and bronchial washings for malignant cells.

The technic of obtaining the specimen follows:

The patient is asked to "cough deeply" and expectorate the mucoid material into a small, sterile jar. It is sent immediately to the laboratory. There, 3 or 4 smears are made, the technician being careful to choose representative portions to place on the slide, i e , one slide is made from any bloody areas, one from the thin, watery material, and one from the thick mucus. After the sputum is spread on the slide, it is immediately placed in the usual fixative of half ether and half alcohol, and stained by Papanicolaou's method. The best sputum sample will be to some degree a mixture of saliva and sputum. It is, of course, essential that real sputum be examined It is of no value whatever to examine saliva for cancer cells from the lung Fortunately, a distinction between saliva and sputum can be made microscopically. Sputum contains phagocytic histiocytes filled with carbon, the "dust" cells Saliva does not contain such cells Therefore, if no phagocytic histiocytes are present, the specimen is unsatisfactory. It is essential that

the specimen actually come from the lower respiratory tract. The clinician can be of great help by carefully instructing the patient.

The cytologic examination of bronchial secretions is extremely satisfactory if care is taken to secure an adequate sample. If, at bronchoscopy, there is a generous amount of secretion, it is aspirated, placed in a sterile tube, and sent to the laboratory, where slides are prepared as for sputum. If very little secretion is present, it may be smeared on a slide immediately and placed in fixative. If there is insufficient material for aspiration, the bronchi should be washed with a small amount of saline solution (2–5 cc.), which is then reaspirated. This is sent to the laboratory, where it is centrifuged, and the sediment is used for the smear. Special attachments for the bronchoscope have been described which facilitate the washing process (50). As with sputum, it is essential that the proper material be obtained. The cytologist is interested in examining material from the bronchus. All too often the material submitted for examination contains only leukocytes and blood and no epithelial cells. Such a specimen should be considered unsatisfactory for examination For the specimen to be adequate, normal epithelial cells, i.e., the ciliated columnar cells from the bronchial epithelium, must be present.

In some cases the bronchial washing, in others the sputum, gives better results In the presence of cancer, the bronchial secretion contains a considerable number of malignant cells. On the other hand, the sputum examination can be repeated easily, as often as desirable, and with no discomfort to the patient. The effectiveness of cytologic specimens removed through the bronchoscope is determined by the interest and care of the physician Unless particular care is taken, the sputum examination may actually be more productive. But some patients with suspicious pulmonary lesions do not have a productive cough The results will be better if both methods are used, rather than either one exclusively

All investigators agree that the use of the cytologic method in the diagnosis of pulmonary carcinoma can measurably increase the number of cases diagnosed. Woolner and MacDonald (84) reported on 75 cases. Of these, the diagnosis by biopsy specimen obtained at bronchoscopy was positive in 41 per cent When the bronchial washings were examined cytologically at the same time, a preoperative positive diagnosis was made in 70 per cent The use of the cytologic method thus increased diagnostic accuracy from 41 to 70 per cent McKay and

Ware (50) reported the same experience in a series of cases in which bronchoscopy yielded a positive diagnosis in 54 per cent, while the result of cytologic examination was positive in 40 of the 54 cases, or 74 per cent. Herbut (35) reported on 540 cases of pulmonary carcinoma. The biopsy specimens were positive in 167 (30.9 per cent). Malignant cells were found in the bronchial secretions in 476 of the same series, or 88.3 per cent.

Thus, in the diagnosis of pulmonary cancer, too, biopsy and the cytologic examination of the bronchial washings are complementary

TABLE 3 —PULMONARY CANCER DIAGNOSED BY CYTOLOGIC SMEAR METHOD

REFERENCE	NO. OF CASES	NO OF PULMONARY CANCERS	% DIAGNOSED ACCURATELY	% POS BIOPSY	% POS. REPORTS CONFIRMED
Dudgeon and Wrigley (13)	—	38	68	—	96
Gowar (25)	—	63	64	—	100
Wandell (80)	193	100	84	—	92.3
Woolner and MacDonald (84)	588	147	70	41	98
Liebow *et al.* (45)	—	49	43	—	88.0
McKay and Ware (50)	170	54	74	54	93.0
Farber *et al.* (17)	1,254	197	55	50	98.2
Jackson *et al* (41)	270	100	61	—	93.9
Herbut (35)	—	540	88.3	30.9	99.6
Watson *et al.* (81)	—	—	69	—	100
Umiker (78)	162	31	70	65	—

methods, and more pulmonary cancers will be recognized when both methods are used concurrently than when either is used alone

The diagnostic accuracy of examination of the sputum and bronchial secretions varies widely, from 43 to 88 per cent (Table 3) This variation is due in a great measure to the method of calculating errors. Some investigators report on multiple specimens and others on single specimens. The results are evidently better in the series in which multiple specimens were used.

[illegible]

normal epithelium is intact over a metastatic lesion.

The false positive results with sputum are low, the figures available in the literature varying from zero to 11 per cent Even with the highest percentage of false positives (11 per cent), in the presence of a posi-

the specimen actually come from the lower respiratory tract. The clinician can be of great help by carefully instructing the patient.

The cytologic examination of bronchial secretions is extremely satisfactory if care is taken to secure an adequate sample. If, at bronchoscopy, there is a generous amount of secretion, it is aspirated, placed in a sterile tube, and sent to the laboratory, where slides are prepared as for sputum. If very little secretion is present, it may be smeared on a slide immediately and placed in fixative. If there is insufficient material for aspiration, the bronchi should be washed with a small amount of saline solution (2–5 cc.), which is then reaspirated. This is sent to the laboratory, where it is centrifuged, and the sediment is used for the smear. Special attachments for the bronchoscope have been described which facilitate the washing process (50). As with sputum, it is essential that the proper material be obtained. The cytologist is interested in examining material from the bronchus. All too often the material submitted for examination contains only leukocytes and blood and no epithelial cells. Such a specimen should be considered unsatisfactory for examination. For the specimen to be adequate, normal epithelial cells, i.e., the ciliated columnar cells from the bronchial epithelium, must be present.

In some cases the bronchial washing, in others the sputum, gives better results. In the presence of cancer, the bronchial secretion contains a considerable number of malignant cells. On the other hand, the sputum examination can be repeated easily, as often as desirable, and with no discomfort to the patient. The effectiveness of cytologic specimens removed through the bronchoscope is determined by the interest and care of the physician. Unless particular care is taken, the sputum examination may actually be more productive. But some patients with suspicious pulmonary lesions do not have a productive cough. The results will be better if both methods are used, rather than either one exclusively.

All investigators agree that the use of the cytologic method in the diagnosis of pulmonary carcinoma can measurably increase the number of cases diagnosed. Woolner and MacDonald (84) reported on 75 cases. Of these, the diagnosis by biopsy specimen obtained at bronchoscopy was positive in 41 per cent. When the bronchial washings were examined cytologically at the same time, a preoperative positive diagnosis was made in 70 per cent. The use of the cytologic method thus increased diagnostic accuracy from 41 to 70 per cent. McKay and

carcinoma could be recognized accurately, but that the accuracy did not meet the optimistic expectations. All too frequently, no malignant cells were present. The accuracy was in general about 50 per cent.

Factors are encountered in the microscopic examination of gastric contents which are not found in other fluids It is true that the examination of each fluid presents peculiar difficulties, but nowhere are they as formidable as in the gastric aspirate. The difficulties are almost entirely technical To obtain a satisfactory specimen of gastric contents which can be used for cytologic examination demands a great deal *more thought, effort, and minute attention to detail than are necessary* for the recovery of specimens from other organs. Were there only one major hurdle to overcome, the problem would be much easier, but unfortunately there are many and varied difficulties.

First, and probably most important, is that in the gastric aspirate, the great majority of the cells seen do not come from the stomach It is difficult to obtain cells from the gastric mucosa The stomach acts as a reservoir for cells being shed and swallowed from the upper gastrointestinal and respiratory tracts. Thus, microscopic examination of the sediment of the gastric secretion may disclose little more than squamous cells, obviously not the cells by which to judge whether a gastric lesion is present or not The problem to be solved, therefore, is how to collect more cells from the stomach itself Papanicolaou, Seybolt, and Cooper (62) have attacked this problem by using a gastric balloon covered with an abrasive net. Panico (55) described the technic in detail

Briefly, the technic consists of aspirating the fasting stomach until it is almost dry At this point a tube is inserted with a balloon attached Marks on the tube indicate the length which should be swallowed. Once in the stomach, the balloon is expanded and carried along by peristalsis The peristaltic action of the stomach on the balloon dislodges cells from the mucosa Cells are scraped off and adhere to the net covering the balloon The air in the balloon is released and the tube is withdrawn Any particles of tissue adhering to the balloon are smeared separately The balloon is then washed in Ringer's solution, the washings are centrifuged, and the sediment is placed on a slide

This attempt to solve the difficulty has certainly produced results as far as the accuracy of the diagnosis is concerned In 152 consecutive cases studied by this method, there were 32 gastric cancers, 24 were called positive, 6 suspicious, and only 2 negative Whether or not this is the final solution of the technical problems is still uncertain It is to

tive cytologic report the possibility of the patient's having cancer is about 90 per cent. Such evidence as this cannot be ignored by the clinician; since it is sometimes difficult to obtain a positive biopsy result by bronchoscopy, surgical exploration is being performed increasingly on the basis of a positive cytologic finding in sputum or bronchial aspirates. McKay and Ware (50) suggested that a positive report should not be the basis for any type of radical surgery, but should certainly be considered as a valid indication for thoracotomy; whether extensive surgery is then performed or not should depend on what is found at the exploratory operation. This would seem to be a reasonable approach to the positive finding in sputum or bronchial aspirate.

Cancer of the lung appears to be increasing, and any method which can be used to facilitate earlier diagnosis is of real value. It is encouraging that Papanicolaou and Koprowska (59), Williams (82), and Umiker (78) have reported cases of carcinoma in situ of the bronchus detected by cytologic examination. If in such early lesions exfoliated cells can be found, there is real hope that systematic use of the microscopic examination of sputum and bronchial washings will disclose lesions while they are still in the easily curable stage. Boucot (6) suggested that any man over the age of 45 either with symptoms or with abnormal x-ray findings be investigated by cytologic study of the sputum. This would seem a reasonable routine. If all methods of early detection are used, it should be possible measurably to increase the survival rate of patients with pulmonary cancer.

GASTRIC CARCINOMA

The diagnosis of gastric carcinoma by means of exfoliated cells has been the subject of mounting interest in recent years The cure rate of gastric cancer is so discouraging that any method which offers hope of discovering the malignant lesion earlier is important and worthy of investigation Soon after the value of the cytologic method in the diagnosis of early cancer of the female genital tract and of the lung was established, attention was focused on the upper gastrointestinal tract

The early reports (32, 38, 58) were devoted to investigations of the feasibility of recognizing malignant cells shed from the epithelial lining of the stomach For the most part, these studies were done on aspirated specimens in the fasting state or on specimens taken after histamine stimulation. It became evident that cancer cells originating in a gastric

is limited by their very specialization. These methods are unfortunately time-consuming for highly trained personnel. Their application to large series of patients is difficult because of this factor. If the cytologic method is to be of real importance in detecting early gastric cancer, it must be applicable to many people with minimal symptoms. This means that simpler technics must be devised which can be performed by less skilled personnel.

The technic in use at the Vincent Laboratory is a simpler one (77).

The fasting stomach is aspirated, this is the first specimen. Then 100 cc. of saline is introduced into the stomach If the patient is ambulatory, he is asked to assume several positions, for example, he on his right side, his left side, stand, sit, etc., in an effort to rinse mechanically all portions of the stomach The saline is aspirated and an attempt is made to recover as much as possible, this is the second specimen Both are taken immediately to the laboratory, centrifuged, and the sediment placed on the three slides which are fixed in ether-alcohol and stained by Papanicolaou's method.

When this type of aspiration was done by the technicians in the laboratory, 84 per cent of the cases of gastric cancer were diagnosed cytologically—an accuracy comparable to that obtained by more elaborate methods However, if the aspirations were performed routinely by house officers, nurses, or doctors in their offices, the accuracy fell to 40 per cent. Imbriglia *et al.* (39) reported 87 per cent accuracy with only a saline wash. This seems to indicate that it may not be necessary to resort to the more complex methods. It is impossible to discount the fact that as soon as experienced laboratory personnel has been responsible for the collection of the specimen, there have been increased positive yields. This principle is common to other technical problems in that the degree of success is proportional to the amount of careful attention applied

There are other considerations than increased numbers of cells shed from the gastric mucosa The enzymes of the stomach cause rapid disintegration of cells once they are removed Time is often the determining factor whether or not a specimen is satisfactory. An interval of more than 30 minutes between the aspiration and the fixing of the specimen is too long. The acid content is an important factor in the problem It may be overcome to some extent by immediate neutralization of the specimen when it is first aspirated In cases of obstruction, it is often almost impossible to secure an adequate sample, due to foreign debris, bacterial contamination, and degenerative changes in the superficial mucosa. All these factors must be taken into consideration,

be hoped that a method may be devised which is less complicated and does not require a physician to perform the test. Though the balloon is not of great size when unexpanded, not all patients find it easy to swallow it. The back of the throat must be anesthetized in some cases to facilitate passage of the tube. An additional factor which will perhaps influence the use of this particular method is the possibility of starting a troublesome gastric hemorrhage by the scraping action of the balloon. Papanicolaou's group state that they have had no serious difficulty in this respect, and that if any amount of frank blood is aspirated, the test is immediately terminated. However, since this possibility does exist, it precludes the test being done by anyone but a physician.

Another attempt to solve this problem has been made by investigators at the University of California Medical School. Rosenthal (70) and his associates have approached the problem in a different fashion. It was their hope that if the mucus in the stomach could be dissolved, there would be an increased natural desquamation of cells. The agent chosen was papain, which was introduced in a buffered solution, left for 10 minutes, and then aspirated Their results were encouraging, in 71 per cent of the cases of gastric carcinoma, malignant cells were recognized. They suggest that one reason the method is an improvement is because the mucolytic action of the papain dissolves a great deal of the debris present and thus makes the slides much easier to read.

Rubin and co-workers (71), at the University of Chicago, have used all these methods to great advantage, chymotrypsin, the balloon described above, and also an antral balloon The last-mentioned is a balloon with a small mercury tip to insure its being carried down to the antrum of the stomach. The results obtained appear to justify the great care taken in this series, and to indicate, as one might expect, that a combination of all methods is superior to any one alone. Rubin reported on 101 cases in which cells from the gastric mucosa were studied. There were 38 cases of adenocarcinoma of the stomach Of these, 31, or 84 per cent, were considered positive cytologically There were 3 sarcomas and 1 case of Hodgkin's disease in the series, and malignant cells were found in all 4 Such figures as these and those of the Papanicolaou group show that by using special technics gastric cancer may be diagnosed with accuracy by cytologic examination

Though such specialized technics as these solve the problem of the low accuracy of cytologic diagnosis of gastric cancer, their usefulness

their gastric contents is not an easy decision Obviously, the clinician must pick and choose the cases rather carefully. The patient with a large gastric neoplasm does not need such an examination, it causes the patient additional discomfort and expense, and is only of academic interest in such cases Whenever possible, if a patient's gastric symptoms warrant a gastrointestinal x-ray series, a cytologic examination should be performed If the early, curable, cases of gastric cancer are to be found, such examination is in order for all patients with minimal symptoms In one group of patients—the pernicious anemia cases—routine gastric aspirations for cytologic examination are essential. This group is known to have a higher incidence of gastric cancer than the general population The cytologic method has proved its value in this group Fifty patients with pernicious anemia have been studied in the Vincent Laboratory. Gastric cancer was diagnosed cytologically in 4, and subsequently confirmed histologically. Of the 4 cases, 2 were unsuspected. In 1 of the four patients, during the 6 months after the positive cytologic report, 4 gastrointestinal x-ray series were negative, a biopsy specimen taken at gastroscopy finally revealed an undifferentiated carcinoma Therefore, it is worth emphasizing that pernicious anemia patients especially should have periodic cytologic examinations of their gastric secretions.

OTHER GASTROINTESTINAL CANCERS

Occasionally malignant cells from tumors not originating in the stomach may be found in the gastric secretion Since the stomach acts as a reservoir for swallowed cells, cells from tumors of the upper alimentary tract may be found. The majority of these are squamous carcinomas of the esophagus. In an investigation (71) of esophageal lesions by cytologic methods, there were 14 patients who clinically were considered to have such lesions. In 9 cases the cytologic findings were called positive, and all of them were proved to have squamous carcinoma The remaining 5 had negative cytologic reports, and were proved to have benign lesions. The accuracy of diagnosis of such lesions seems great, especially if an attempt is made to aspirate cells directly from the esophagus Fortunately, the cells of squamous carcinoma are so characteristic that the cytologist can identify the tumor cells in most instances as originating from squamous rather than columnar epithelium

Interest in the use of cytologic methods for diagnosis of malignant

and it is obvious that the interested personnel of the cytology laboratory can deal with them most effectively.

The effectiveness of the method varies between 50 and 85 per cent, depending on how the specimen is collected, and, more important, on who collects the specimen. The stimulus for this type of approach to the early diagnosis of gastric cancer is the fact that early cancers have been discovered. At the Vincent Laboratory, 4 cases of gastric carcinoma in situ have been found by cytologic investigation

The high curability of such early lesions and the bleak prognosis for the clinically detectable lesion make cytologic study of particular importance in this area. It has certain obvious limitations If a scirrhous tumor of the stomach is present, the surface epithelium is usually not involved and the cytologic examination will give negative results. Gastric lymphoma is infrequently diagnosed cytologically.

The number of false positive results reported in cytologic studies of the stomach is by no means prohibitive. If calculated against the positive cases, they vary between 4 and 11 per cent (62, 66, 71, 77), so that the possibility of cancer when the report is positive is over 90 per cent. Such evidence cannot be ignored. It compares favorably with the accuracy of roentgenography. In the first 100 cases studied by the balloon method, Papanicolaou *et al* (62) reported that of the 75 patients with proved benign lesions, 11, or 14 per cent, had been suspected of having cancer on the basis of roentgenographic findings In the same series of benign lesions, there was only 1 false positive cytologic report.

For the clinician, the problem presented by repeated positive gastric cytology findings in a patient without other definite signs or symptoms of malignancy is a serious one. If the report comes from a laboratory with experience in this field, immediate investigation by a repeat gastrointestinal x-ray series and gastroscopy is called for. Even when this evidence is negative, repeated positive gastric washings are a valid indication for an exploratory laparotomy Further procedures then depend on what is found at operation.

If gastric cancer is to be diagnosed in earlier stages, the patient in whom the repeated gastric cytologic examination has been positive cannot be watched until the lesion has grown large enough to produce characteristic shadows on the roentgenogram There is no doubt that if a positive gastrointestinal x-ray series is considered to be an indication for an exploratory operation, a report of positive cytology has equal validity.

Which patients should be submitted to a cytologic examination of

patients in whom the prostatic secretion obtained by massage had been examined for tumor cells; 66 had cancer of the prostate, and malignant cells were found in 54—an accuracy of 81.8 per cent; these cases had been recognized clinically as carcinomas of the prostate. His later report (37) deals with the use of the method as a screening procedure in clinic patients; not a single case of occult carcinoma of the prostate was discovered in this series. We have had the same experience at the Vincent Laboratory, although unsuspected cancer has been found in every other site investigated, only once has an unsuspected prostatic cancer been discovered by cytologic examination.

Peters (64) regards the method as accurate and worthy of extensive use. *In 100 cases of cancer of the prostate, the prostatic smear was positive in 88, suspicious in 7, and negative in 5. Of the cases in which cancer cells were found, 5 were considered to have been discovered earlier* than would have otherwise been possible Peters emphasizes that the *specimen must be adequate. The secretion obtained by prostatic massage* is placed directly on a glass slide, spread by overlaying a second *slide*, and fixed *immediately*

In carcinoma of the bladder, cancer cells can be found in about 65 per cent of the cases by *examination of the urinary sediment* (8) *The* accuracy, therefore, is similar to that of sputum examination, and, as with sputum, repeat *examinations will substantially increase the* number of correct diagnoses Early, unsuspected carcinoma of the bladder may be discovered Chute and Williams (8) reported a case in which smears of the urinary sediment were consistently positive, whereas a positive biopsy specimen was not obtained until the eighth cystoscopic examination.

Though Herbut (37) reports that he has never seen cancer cells in the urine from a patient with renal carcinoma, the experience at our laboratory has been quite different· of 18 patients with renal tumors, cancer cells were present in the urine of 9, and 3 unsuspected cases were discovered Papanicolaou (56) has reported a case of in situ carcinoma of the renal pelvis discovered by examination of the ureteral urine specimens obtained at cystoscopy It is encouraging to know that desquamated cancer cells were found in such an early case

There are two troublesome factors in the diagnosis of tumors of the urinary tract One difficulty is that so many of the specimens are unsatisfactory because there are so few cells to examine. The amount of sediment after centrifugation of either a voided or a catheterized specimen of urine is often inadequate Deden (12) attempted to overcome

lesions of the bowel has not been great, this is surprising since its application is simple and the accuracy high. Bader and Papanicolaou (5) *have studied rectal or colonic washings of 200 patients. To prepare* the patients, 2 oz. of castor oil was administered the night before the test, and the morning of the test, the patient received a cleansing enema. The rectal washing was carried out in conjunction with the proctoscopic examination. Loeb and Scapier (46) have devised a special instrument to facilitate collection of the material. Essentially, saline is introduced and then collected by a test tube introduced into the aspirating system as a trap. If the suspected lesion is beyond the reach of the proctoscope, 500 to 800 cc. of normal saline is introduced as an enema and collected. After collection, the material is centrifuged and the sediment is smeared, fixed, and stained as usual.

The results from this method are encouraging. In the 200 cases *studied by Bader* [illegible] of the rectum or [illegible] of the washing, 4 suspicious, and only 1 negative. Malignant cells were found in 1 case 6 months before a positive biopsy result could be obtained. In 1 case not included in their series, a carcinoma of the cecum, malignant cells were found in the bowel washings This seems to indicate that the method has value in the diagnosis of cancer located in any part of the large bowel Rubin (71) reported finding cells in a case of sarcoma of the duodenum Of 32 cases of carcinoma of the colon, he found malignant cells in 26, an 82 per cent accuracy. There were no false positives Such figures strongly suggest that the application of the cytologic method to the diagnosis of cancer of the bowel is well worth while. It is not as successful in carcinoma of the biliary tract or pancreas, though cases of pancreatic cancer discovered by cytologic examination of duodenal drainage material have been reported by McNeer and Ewing (51).

URINARY TRACT CANCER

Use of the cytologic method in the diagnosis of urinary tract malignant tumors has largely been focused on the diagnosis of prostatic cancer. Obviously, if it could be used here with the same results as in other sites, the method would be of real importance in detecting early and potentially curable cases Unfortunately, the reports are conflicting Herbut, who originally (36) was very enthusiastic about the method, *has since* lost much of his enthusiasm (37). In 1949 he reported on 480

sible cancer cells in serous fluids. As in all cytology, the negative report does not rule out the possibility of cancer, but the positive report is strong evidence that a malignant lesion is present.

Sattenspiel (74), comparing the accuracy of the sectioned "button" and the smear of serous fluid sediment, found that the results of the smears were superior to those of the older method. This was especially true when there was little sediment. He concluded that results were better when both methods were used concurrently.

Breast Secretions

Cancer of the breast is the most common malignant tumor encountered in women. Recently, attempts have been made to discover early cases by cytologic examination of the secretion from the nipple.

The technic of obtaining a specimen is simple Any spontaneous secretion present is [illegible]
is immediately fixed [illegible]
logic specimen. Sap[illegible]
entirely limited to patients with spontaneous secretions However, in 3,013 patients, Jackson *et al.* (40) obtained secretion in 50 per cent by gentle massage of the nipple, they emphasized that the massage should be gentle and nontraumatic

The cytologic study of breast secretion is not limited to the diagnosis of mammary cancer It is also possible to diagnose chronic cystic mastitis and papilloma. In cases of chronic cystic mastitis the secretion contains characteristic phagocytes with ingested lipid material Of 21 cases diagnosed by Saphir as chronic cystic mastitis, 18 were proved to have the disease by histologic examination. Of 13 cases considered to be either positive or suspicious for papilloma of the breast, 8 were proved to be so The cytologic picture of papilloma consists of clusters of small epithelial cells and macrophages containing blood pigment Jackson reported that 46 per cent of the cases of silent papilloma were detected by cytologic examination of breast secretions before any definite symptoms had occurred. Since papilloma of the breast is considered to be a premalignant lesion, the fact that such cases can be discovered by examination of the nipple secretions is important

Of 18 cases of cancer of the breast in which the secretions were examined by Saphir (73), cells which either aroused suspicion or gave conclusive evidence for the presence of carcinoma were found in 15, 3 cases considered positive or suspicious for carcinoma were subse-

this difficulty by adding equal parts of 95 per cent alcohol to the specimen, placing the fluid in a separatory funnel, and allowing it to stand overnight. Much technical work remains to be done in securing adequate samples for examination.

The second difficulty is the frequency of false positive reports. Chute and Williams reported that 70 per cent of their positive reports could be confirmed. Though this figure is reliable enough to warrant investigation of any patient who has a positive report, it should be remembered that a report on urine sediment does not have the same specificity as in other types of secretions. However, if the method is used with these points in mind, unsuspected early cases of carcinoma of the kidney and of the bladder can be discovered.

CYTOLOGIC EXAMINATION OF OTHER FLUIDS AND SECRETIONS

Serous Fluids

Serous fluids have been examined for the presence of malignant cells for many years Quensel (67), in 1921, described tumor cells identified in ascitic and pleural fluids. This method has been in general use in pathology laboratories and actually represents the first practical use of the cytologic method for the diagnosis of malignancies. The difference is mainly technical The technic in general use is that of centrifuging the specimen, embedding the "button" in paraffin, then sectioning the material in the usual way In recent years, more laboratories have come to prefer the simpler technics used in the cytologic laboratory The fresh fluid is centrifuged, the sediment placed on a slide, fixed, and stained as for any other type of secretion. If the material is particularly bloody or has many leukocytes, the malignant cells may be layered out by adding different concentrations of albumin to produce a differential in specific gravity (18) so that the malignant cells concentrate in one layer

Saphir (72) has reported on a series of 175 specimens of ascitic or peritoneal fluid examined by the cytologic method Of these, 45 were from subsequently proved carcinomas of the pleura or peritoneum In 37, or 82 per cent, the cytology report was positive, 6 were called suspicious, and the remaining 2 negative (4 per cent) There was one false positive report (2 6 per cent) The positive reports on fluids are extremely accurate and few false positive reports are returned The cytologist has to be extremely conservative in the interpretation of pos-

specimen was 98 per cent. This method enables the surgeon to know fairly rapidly whether or not the tumor has involved the nodes

Cytologic diagnosis of malignant disease is expanding rapidly. It can be used for any site from which cells may be collected. It began as a study of exfoliated cells only, but, as lymph node studies indicate, it now includes other types of examination. Increasingly wider application of this diagnostic method seems most probable, and along with it the discovery of morphologic changes in single cells in pathologic states other than cancer.

CONCLUSIONS

The cytologic method of diagnosing cancer by exfoliated cells has obviously established its usefulness With every fluid examined, cases of early, symptomless cancers have been discovered. The accuracy of the cancer diagnosis is good, the false positive results decreasing as greater experience in the interpretation of exfoliated cells is gained.

The method, however, is subject to certain limitations. First, and most important, is that a negative report does *not* exclude the possibility of cancer. Second, a small number of results are false positives; corroborative evidence should therefore be obtained before any definitive treatment is instituted. If a biopsy can be performed easily, as in the case of uterine cancer, it should be done before starting treatment; if the result is negative, the biopsy should be repeated Even if the site does not readily lend itself to biopsy, it should be attempted nevertheless: for example, bronchoscopic biopsy in cases of positive sputum findings, or gastroscopic biopsy when gastric secretion smears are positive. If the biopsy specimen is negative and the cytologic examination is repeatedly positive, exploratory surgery is indicated, further treatment will then depend on what is found at operation

carc

to t

the vaginal secretions may be exfoliating from an adenocarcinoma of the cervix, endometrium, tube, or ovary. Furthermore, it is impossible to tell with any degree of accuracy whether a lesion is still in situ. *These* answers must *still* be made by the histologic examination of the tissue in question.

The accuracy of the method depends on the experience of the cytologist, but of equal importance is the technic of collecting the

quently proved to be either chronic cystic mastitis or papilloma. This accuracy compares favorably with that of the cytologic examination of other secretions.

As in other types of cancer in which the cytologic method has been applied, unsuspected cases of breast cancer have been discovered by *examination of secretion from the nipple*(14, 42). *There is no doubt* that this type of examination is of value in deciding the underlying pathology in patients with bleeding nipples.

Although this particular cytologic examination is rather new, it apparently has already established its usefulness. The accuracy is comparable to that obtained with other secretions. Not only early cases of cancer may be discovered; at the same time, the characteristic cells desquamating from chronic cystic mastitis and papilloma of the breast render the diagnosis of these lesions possible.

Secretions from Nose and Throat

The study of single exfoliated cells is gradually being extended to other sites of malignancy than the ones discussed above. Friedmann (22) reported dependable and encouraging results in diagnosis of tumors of the nose and throat. Fitzhugh *et al.* (19) used washings from the maxillary sinus and were able to diagnose 6 cases of carcinoma

Lymph Node Smears

One interesting application has been the study of lymph node smears or impressions Although they were studied by several investigators in the past, the renewed interest in single cell morphology has caused an increased awareness of the possible usefulness of lymph node impressions Moore and Reagen (52), in a series of lymph node imprints, found distinct cellular morphology in patients with lymphoma and metastatic carcinoma Malignant cells were found in imprints of nodes in which the presence of tumor was proved by histologic section

Dearing (11) has used smears of nodes, at operation for carcinoma of the vulva, in order to decide immediately whether to proceed with a radical lymph node dissection Half of the node was used to make a smear which was fixed, stained, and examined at once, the other half was fixed for histologic examination The agreement between the diagnosis on the basis of the lymph node smear and that of the histologic

15 Ellis, F. H., Woolner, L. B., and Schmidt, H. W.: Study of factors involved in exfoliation of malignant cells, J. Thoracic Surg 20 125, 1950
16 Erickson, C. C., *et al*: Preliminary report of 20,000 women studied by vaginal smear technique in a general population cancer screening project, Proc. Am A Cancer Res 1·14, 1953.
17. Farber, S. M., *et al*: *Cytologic Diagnosis of Lung Cancer* (Springfield, Ill.: Charles C Thomas, Publisher, 1950)
18 Fawcett, D. W., *et al*: A method for concentration and segregation of malignant cells from bloody pleural and peritoneal fluids, Science 3·34, 1950
19 Fitzhugh, G. S.; Moon, C. N., and Luptom, C. H., Jr.. Cytologic smear technique in diagnosis of carcinoma of maxillary sinus, Laryngoscope 60 376, 1950
20 Foote, F. W., and Li, K. Smear diagnosis of in situ carcinoma of the cervix, Am J. Obst. & Gynec 56 335, 1948.
21 Frech, H. C: Adenocarcinoma of ovary diagnosed by vaginal smear, Am J. Obst. & Gynec. 57:802, 1949.
22 [illegible] diagnosis of tumours of [illegible]
23 [illegible] or cervical cancer in internist's office by routine vaginal smear, J.A.M.A. 150 587, 1952
24 Gates, O., and Warren, S. *A Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smears* (3d ed., Cambridge, Mass.: Harvard University Press, 1949)
25 Cowar, F. J. S. Carcinoma of the lung. The value of sputum examination in diagnosis, Brit J Surg 30 193, 1943
26 Graham, J. B., and Meigs, J. V. Earlier detection of recurrent cancer of the uterine cervix by vaginal smear, Am J Obst & Gynec 64 908, 1952
27 Graham, R. M. The effect of radiation on vaginal cells in cervical carcinoma, Surg, Gynec. & Obst 84 153, 166, 1947
28 Graham, R. M. The prognosis of cancer of the cervix by vaginal smear. Correlation with five-year results, Surg, Gynec. & Obst 93 767, 1951.
29 Graham, R. M. The value of the vaginal smear to the gynaecologist, J Obst & Gynaec Brit Emp 60 483, 1953
30. Graham, R. M., and Graham, J. B. A cellular index of radiation sensitivity, Cancer 6 215, 1953
31 Graham, R. M., Sturgis, S. H., and MacGraw, J. A comparison of the accuracy in diagnosis of the vaginal smear and the biopsy in carcinoma of the cervix, Am J Obst & Gynec 55 503, 1948
32 Graham, R. M., Ulfelder, H., and Green, T. H. The cytologic method as an aid in the diagnosis of gastric carcinoma, Surg, Gynec & Obst 86 257, 1948
33 Gusberg, S. B., and Graham, D. A. Development of the vaginal cytology laboratory. Its precision and significance, Am J Obst & Gynec 59 1053, 1950
34 Haynes, D. M. Cytological methods for early detection of uterine cancer, J A M A. 148 457, 1952
35 Herbut, P. A. Correlation of Cytological with Pathological Findings in Tumors of the Lung, in *Proceedings, Symposium on Exfoliative Cytology, 1951* (New York: *American Cancer Society*, 1953), p 50

material. If the clinician wishes to use the method to the best advantage, it is wise to find out from the laboratory exactly how the specimen should be collected and to attempt to follow the procedure as closely as possible.

The rapid expansion of the cytologic method to many different types of tumor has established the method as useful wherever exfoliated cells may be collected. The diagnosis of cancer by the study of exfoliated cells has become established as an important method in the diagnosis of malignant disease.

REFERENCES

1 Achenbach, R R, Johnstone, R. E., and Hertig, A.: The vaginal smear diagnosis of carcinoma in situ, Am. J. Obst & Gynec. 61·385, 1951
2. Anderson, A. F., Grant, M. P. S., and McBridge, R.: Diagnosis of cancer of the uterus, Proc Royal Soc. Med. 44 308, 1951.
3. Ayre, J E.· Selective cytology smear for diagnosis of cancer, Am J Obst & Gynec 53 609, 1947
4. Ayre, J E Vaginal cell examinations as a routine in diagnosis, South M J 39 847, 1946.
5 Bader, G M, and Papanicolaou, G. N The application of cytology in the diagnosis of cancer of the rectum, sigmoid, and descending colon, Cancer 5.307, 1952
6 Boucot, K The value of cytological aspect in screening Lung aspects, in *Proceedings Symposium on Exfoliative Cytology, 1951* (New York American Cancer Society, 1953), p. 120.
7 *Burns, E L., Hartung, W H, Jr, and Brittingham, E Studies of early* uterine carcinomas discovered by cytological examination of vaginal contents, Arch Path 50 699, 1950
8 Chute, R, and Williams, D. Experiences with stained smears of cells exfoliated in the urine in the diagnosis of cancer in the genito-urinary tract: A preliminary report, J Urol. 59 604, 1948
9 Cuyler, W K., *et al.* Genital cytology in obstetric and gynecologic patients, Am J Obst. & Gynec 62 262, 1951
10 Cuyler, W K The Recognition of Carcinoma in situ of the Cervix Uteri by Papanicolaou's Method, in McManus, J F A (ed). *Progress in Fundamental Medicine* (Philadelphia Lea & Febiger, 1952)
11 Dearing, R Diagnosis of malignant involvement of lymph nodes by smear technique, J Obst & Gynaec Brit Emp 59 385, 1952.
12 Deden, C. The demonstration of cancer cells in the urine as a diagnostic aid Acta radiol 37·485, 1952
13 *Dudgeon, L S, and Wrigley, C H On the demonstration of particles of* malignant growth in sputum by means of wet-film method, J Laryng & Otol 50 752, 1935
14 Eisen, M F, and Taft, R Cytological diagnosis of mammary cancer associated with incipient Paget's disease of the nipple, Cancer 4 150, 1951

57. Papanicolaou, G. N.. A new procedure for staining vaginal smears, Science 95.438, 1942
58. Papanicolaou, G N., and Cooper, W. A . The cytology of the gastric fluid in the diagnosis of cancer of the stomach, J Nat Cancer Inst. 7·357, 1947.
59. Papanicolaou, G. N , and Koprowska, I.: Carcinoma in situ of the right lower bronchus, case report, Cancer 4:141, 1951.
60 Papanicolaou, G N , and Marchetti, A. A . Use of endocervical and endometrial smears in diagnosis of cancer and other conditions of the uterus, Am. J. Obst. & Gynec 46.421, 1943
61. Papanicolaou, G N , and Traut, H F · *Diagnosis of Uterine Cancer by the Vaginal Smear* (New York: Commonwealth Fund, 1943)
62 Papanicolaou, G N.; Seybolt, J , and Cooper, W. A . Value of gastric smears in the early diagnosis of gastric cancer, in *Proceedings, Symposium on Exfoliative Cytology, 1951* (New York· American Cancer Society, 1953), p. 95
63 Parsons, L., and Taymore, M Carcinoma of the breast metastatic to the peritoneum as a source of positive vaginal smears, Am J. Obst. & Gynec 66.194, 1953.
64 Peters, H The cytologic interpretation of the prostatic smear, Surg , Gynec & Obst. 94.69, 1952
65 Peters, H , and Madden, W. L The cervical smear in office patients, J A M A 142 624, 1950
66 Porter, J , *et al* Differential diagnosis of gastric lesions from cells obtained by stomach aspiration, Maine M. A J. 42 67, 1951.
67 Quensel, U. Über die Moglichkeit Geschwulstzellen in den Ergussen der serosen Hohlen nachzuweisen, Acta med scandinav 53 765, 1921
68 Reagen, J W , and Schmidt, R T Evaluation of cytological technic in recognition of malignant uterine neoplasms, J A M A 145 82, 1951
69. Reichter, N B , *et al* Clinical evaluation of 3500 vaginal cytologic studies, Am J Obst & Gynec 59 860, 1950.
70 Rosenthal, M Use of papain as a mucolytic agent in procuring gastric smears, in *Proceedings, Symposium on Exfoliative Cytology, 1951* (New York American Cancer Society, 1953), p 89
71. Rubin, C , *et al* The clinical value of gastrointestinal cytologic diagnosis, Gastroenterology 25 119, 1953.
72. Saphir, O . Cytologic diagnosis of cancer from pleural and peritoneal fluids, Am. J. Clin. Path 19 309, 1949
73. Saphir, O Cytologic examination of breast secretions, Am J Clin Path. 20 1001, 1950
74 Sattenspiel, E Cytological diagnosis of cancer in transudates and exudates A comparison of the Papanicolaou method and paraffin block technique, Surg , Gynec & Obst 89 478, 1949
75 Sheffey, L C , Rakoff, A E , and Hoffman, J An evaluation of the vaginal smear method for the diagnosis of uterine cancer, Am J Obst & Gynec 55 453, 1948
76 Skapier, J Evaluation of cytologic test in early diagnosis of cancer Two year survey of routine use of the smear technique, Am J Obst & Gynec 58 366, 1949

36 Herbut, P. A. Cytologic diagnosis of carcinoma of the prostate, Am. J Clin Path. 19 315, 1949.

37. Herbut, P. A.: *The value of the cytologic techniques in the diagnosis of lesions of the genitourinary tract*, in *Proceedings, Symposium on Exfoliative Cytology, 1951* (New York: American Cancer Society, 1953), p. 61.

38 Hunter, W. C., and Richardson, H. L: Cytologic recognition of cancer in exfoliated material from various sources, Surg, Gynec. & Obst. 85 275, 1947.

39 Imbriglia, J.; Stein, G N., and Lopusniak, M S · Cytological study of upper gastrointestinal sediment, J A.M.A, 147:120, 1951.

40. Jackson, D, Todd, D. A, and Gorsuch, P. L: Study of breast secretion for detection of intramammary pathologic changes and of silent papilloma, J Internat Coll. Surgeons 15.552, 1951.

41. Jackson, E., Bertoli, F., and Ackerman, L.: Exfoliative cytology. An adjunct in the diagnosis of bronchogenic carcinoma, J. Thoracic Surg 21:7, 1951.

42. Jacobsen, B D.: Early diagnosis of carcinoma of breast by cytologic technique with report of a case, New Jersey M. Soc. J. 47.337, 1950.

43. Jones, C A, Neustaedter, T., and MacKenzie, L. L.· Value of vaginal smears [illegible] c. 49 159, 1945.

44 [illegible] sis of malignant disease [illegible] 1950

45. Liebow, A. A., Lindskog, G. E., and Bloomer, W. E.: Cytological studies of sputum and bronchial secretions in the diagnosis of cancer of the lung, Cancer 1·223, 1948

46. Loeb, R., and Scapier, J Rectal washings, technic for cytologic study of the rectosigmoid, Am J Surg 81 298, 1951.

47 Lombard, H, *et al* Use of the vaginal smear as a screening test, New England J Med 246 523, 1952.

48. MacCarty, W C Has the cancer cell any differential characteristics? Am J Cancer 20 403, 1934

49. MacLean, K S Tubal malignancy—a method of collecting specimens for cytologic study, Science 114 181, 1951.

50 McKay, D G., and Ware, P The diagnosis of bronchogenic carcinoma by smears of bronchoscopic secretions, Cancer 1 208, 1948

51 McNeer, G, and Ewing, J H Exfoliated pancreatic cancer cells in duodenal drainage, Cancer 2 643, 1949

52 Moore, R D, and Reagen, J A cellular study of lymph node imprints, Cancer 6 606, 1953

53. Nieburgs, H. E, and Pund, E. R Detection of cancer of the cervix uteri Evaluation of comparative cytologic diagnosis, study of 10,000 cases, J A M A 142 221, 1950.

54. Nielsen, A M Cytological changes in vaginal smears in radium and roentgen irradiation of uterine carcinoma and their prognostic significance (preliminary report), Acta radiol 37 479, 1952.

55. Panico, F. G Improved abrasive balloon for diagnosis of gastric cancer, J A M A. 149:1447, 1952.

56. Papanicolaou, G N. Cytologic diagnosis of uterine cancer by examination of vaginal and uterine secretion, Am J Clin Path 19 301, 1949

Spatial Vectorcardiography*

ARTHUR GRISHMAN†

Assistant Attending Physician for Cardiology, The Mount Sinai Hospital, New York

MANY ADVANCES have been made in the theory and application of electrocardiography since Waller (85) reported, in 1887, the first successful recording of the electromotive changes associated with the action of the human heart. Einthoven's epochal contribution of a string galvanometer of great fidelity, which allowed the accurate recording of action potentials, made possible meaningful experimentation and testing of theories. The following decades saw numerous improvements and simplifications in technical apparatus, although not always in their accuracy (48, 66). For more than 20 years the use of the three bipolar extremity leads has formed the basis of clinical and experimental investigations In this interval, little has been added to our knowledge of the rhythmic properties of the heart and its disturbances What Lewis (56) had done for the analysis of the arrhythmias, Wilson (92, 94, 96) similarly accomplished for the ventricular complex of the electrocardiogram His important contributions, consisting of an analysis of precordial potentials in patients and their elucidation by appropriate animal experiments, and of the introduction of the central terminal electrode system for precordial and extremity electrocardiography (95) led to assiduous accumulation and study of case records

* Aided by grants from the Dazian Foundation for Medical Research, the Jacob Sachs Medical Research Foundation, and the Enandros Research Foundation

† The author acknowledges with appreciation the efforts and contributions of his co-workers, past and present E R Borun, R P Lasser, L Scherlis, and F Donoso

77. Ulfelder, H.; Graham, R M, and Meigs, J. V.: Further studies on the cytologic method in the problem of gastric cancer, Ann. Surg. 128 422, 1948
78. Umiker, W.: Bronchogenic carcinoma in situ, Cancer 5:369, 1952
79. Umiker, W.: Cytology in bronchogenic carcinoma, Am. J. Clin. Path 22 558, 1952.
80. Wandall, H. H.: A study on neoplastic cells in sputum as a contribution to the diagnosis of primary lung cancer, Acta chir. scandinav. Supp 93, 1944
81. Watson, W. L., *et al*: Cytology of bronchial secretions, J Thoracic Surg 18:113, 1949.
82 Williams, M. J.: Extensive carcinoma in situ in the bronchial mucosa associated with two invasive bronchogenic carcinomas, Cancer 5 740, 1952
83. Woolner, L. B, and MacDonald, J. R: Carcinoma cells in sputum and bronchial secretions, Surg, Gynec. & Obst. 88 273, 1949.
84. Woolner, L. B, and MacDonald, J R.: Cytology of sputum and bronchial secretions· Studies on 538 patients with miscellaneous lesions, Ann. Int Med 33.1164, 1950.

potentials" to the electrocardiogram, since it is believed that such potentials are not available to instrumental analysis with any of the electrocardiographic technics employed.

THEORY OF VECTORCARDIOGRAPHY

The "vector concept" underlying spatial vectorcardiography assumes that the generation of electromotive forces by all segments of the active heart contribute to one electromotive field. All electrocardiographic leads, irrespective of the technic employed, merely "tap" this field through projection upon lead lines, the angle of which is determined by their respective points which are connected to the galvanometers. It can be readily seen that the unitarian character of the vector concept tries to treat all electrocardiographic curves obtained from the same individual as direct derivations of one source common to all of them. The return to one of Einthoven's theories is apparent: the electric forces generated by the heart can be presented as a dipole at the center of a sphere with essentially homogeneous conductivity

The following pages present the principles of the vector concept, discuss the dipole and vector principles underlying it, and describe technics for electrode placement and for recording the spatial cardiac vector. The clinical value of the method, its present position as a clinical diagnostic tool, and its impact on the teaching of electrocardiography, are briefly summarized

Dipole Theory

The fundamental theoretic basis of spatial vectorcardiography is the dipole theory, as proposed by Einthoven *et al* (23) and further developed and reviewed, experimentally and theoretically, by various investigators (9, 10, 12, 15, 16, 28, 54, 60, 77, 78, 94) The basic tenet of the dipole theory holds that the electric field which is generated by cardiac activity behaves as though it were produced by a simple battery with positive and negative poles close together, a battery which is immersed in a homogeneous volume conductor of body tissues and fluids. The positive and negative poles constitute a dipole and are actually formed in the body by the relationship of the positively and negatively charged ions of tissue fluid electrolytes. The manner in which forces are generated by the stimulated muscle cell and through which the dipole relationship between ions is created and maintained

and to painstaking collection of and correlation with autopsy material (61–63).

Electrocardiography has become such an essential part of clinical medicine that without it no cardiac examination can be regarded as complete. Although the diagnostic yield of clinical electrocardiography is great, more accurate information about the electric properties of the functioning myocardium is still needed. Standard technics may not give evidence of myocardial infarction, as indicated by clinical evidence or revealed on postmortem examination. In other instances it may be difficult or impossible to differentiate intraventricular conduction defects and bundle-branch block from those of ventricular hypertrophy or even from those due to myocardial infarction The attempts to obtain additional information by means of exploring electrodes in nonstandard positions yielded little. Esophageal, intracardiac and multiple thoracic leads (in nonstandard positions) have been employed, but the technics were cumbersome, the results proved confusing or of limited value, and often only added to the discomfort of the patient. As Wolff and co-workers (97) recently stated: "Electrocardiography as a method of examination has been thoroughly explored, and it is unlikely that its usefulness will be extended to any important degree"

Electrocardiography with multiple exploring leads is based on the concept that the leads are able to record predominantly the electric activity of a circumscribed myocardial area subjacent to the exploring electrode, i e., localized potential differences This concept assumes that the different anatomic areas of the ventricular myocardium have electrocardiographic patterns which are essentially characteristic of the specific area. The assumption was based on animal experiments in which thoracic electrocardiograms had a configuration identical with those obtained from the myocardial surface subjacent to it (92, 96) The former, therefore, have often been referred to as "semidirect" leads because of their similarity to the latter, the "direct" leads. Although the facts established by the experiments cannot be denied, other explanations can be offered for them as a result of changing concepts and additional experience. Thus, the concept of exploring *electrocardiography led to the search for the location of characteristic* patterns on the chest wall and extremities, within the large thoracic vessels, in the ventricular cavities and esophagus The only limiting factor in this type of investigation is the accessibility of the area to electrode exploration. In recent years, there has been a tendency to minimize the importance of the contributions made by specific "local

"elementary" dipole contributes to the magnitude, direction, and sense of the total or resultant electromotive force at the instant of observation; this might be termed the balance of forces. Therefore, at any one instant one dipole, the resultant of all "elementary" dipoles, and one vector, the resultant of all elementary vectors, can be used to represent the characteristics of the cardiac electromotive field force within the body. The resultant vector is termed the instantaneous cardiac vector. The geometric structure inscribed by the (to be imagined) arrowheads of all instantaneous cardiac vectors during an entire cycle of cardiac activity is termed the cardiac vector (Fig 1).

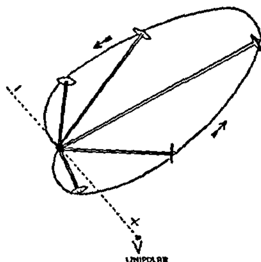


FIG 1 –The arrowheads of all instantaneous vectors form the vector loop.

The heart and its active muscle segment have a three-dimensional arrangement. The instantaneous vectors representing the magnitude, direction, and sense (polarity) of the electromotive field forces of each moment will correspondingly have a three-dimensional or spatial orientation, i e., left or right, superior or inferior, anterior or posterior. A spatial cardiac vector loop is inscribed by the terminal points of all instantaneous vectors throughout one cardiac cycle (Fig. 2). The loop inscribed by atrial activity is termed the P loop, that produced by depolarization of the ventricular muscle mass, the QRS loop, and the one inscribed by the repolarization process, the T loop. From the cardiac vector loop one can derive information concerning the balance of forces contributed by the muscle aspects at any one time.

is not relevant to a discussion on spatial vectorcardiography which will be limited to the validity of the dipole concept, its properties, and its recording (instrumental analysis).

An excited cardiac muscle area will assume a negative charge in relation to a resting one, which, therefore, becomes relatively positive Thus, through the apposition of a negative and positive pole in close proximity to one another, a dipole is created. Like the creation of a magnetic field through the north-south orientation of a magnet, the newly created dipole creates an electric field around it. A line drawn through the two poles of the dipole constitutes the dipole axis. The direction of this line, determined by the spatial orientation of its two poles to each other, indicates the direction of positive and negative electricity in the field, and hence the direction of the current flow The dipole center is the point of zero potential between the two components of the dipole. The dipole axis is more familiarly known as the electric axis of the heart, so named by Einthoven, *et al.* (23). The magnitude of the dipole charge at the time of observation, or the dipole moment, is shown by giving it a distance, specified by standardization, as expression of the voltage generated. Transformation of voltage into a displacement of a specified magnitude allows the graphic presentation of voltage (1 cm. displacement may represent 1 mv.) Recording on a kymogram or a recorder running at a known speed produces a record of the voltage and its time of occurrence in relation to other events

The appearance of a dipole creates an electromotive force, and, having the properties of magnitude, direction, and sense, it is a vector quantity. In contradistinction to so-called scalar quantities, such as mass, volume, and potential difference which have no reference to direction, vector quantities are subject to the mathematical rules governing vector addition and can be visualized through vector presentation

The mathematical symbol which represents a vector quantity is an arrow The direction of the arrow indicates the direction of the electromotive force created by a dipole in space, the length of the arrow indicates the magnitude of the force, and the head of the arrow points in the direction of the positive field, thereby following Einthoven's choice of polarity Vector presentation, therefore, can express in a single symbol the three properties of a dipole: magnitude, direction, and sense (polarity).

At every instant of cardiac activity myriads of minute or "elementary" dipoles arise, each of which develops an elementary electromotive force which can be represented by an "elementary" vector Each

Spread of the Excitation Wave

The sequence of excitation of the ventricular musculature and the generation of electromotive forces following the excitation are determined by the anatomy of the bundle of His and the Purkinje fibers.

The study of galvanometric deflections from leads at or near the heart can yield only general information as to the order of the spread of the excitation wave, since these are merely recording points in the field of the resultant dipole (26). Better understanding of the various lead systems (unipolar, semiunipolar, distant electrodes paired with exploring electrode, etc.) upon which such information was based makes doubtful the exactitude ascribed to the experimentally obtained data (21, 22, 35, 38, 60, 76). On the basis of present knowledge about the physical properties of the lead systems formerly employed in such experiments, Fahr's (26) statement of 1920 that the information is general and devoid of the exactitude implied, is completely justified. The curves obtained "tapped the spatial cardiac vector," i e., the resultant of all forces generated and not predominantly the electrical activity of the muscle area subjacent to the exploring electrode. Lewis *et al.* (57) were aware of the limitations of the technic employed as early as 1914, but in subsequent years this aspect was minimized. Harris's (41) experiments and, more recently, those of Schaefer (69) with "micro" bipolar systems, with the two electrode points as close to each other as possible, have yielded sufficiently interesting data to warrant more investigations with the same or similar technics.

The heart can be considered to be a spherical shell with a septum dividing it into two chambers The stimulus appears to reach the muscle fibers of the anterosuperior segment of the septum first. The balance of forces at this time is oriented so that the vector of the septal balance of forces is directed anteriorly and to the right Thereafter, many segments of the septum and ventricular walls are stimulated simultaneously, and only the sum or net balance of all forces generated at any time is then available to instrumental analysis in man.

SPATIAL VECTORCARDIOGRAPHY

The spatial cardiac vector, inscribed by all instantaneous cardiac vectors, is believed to contain all the information about the field strength—and thereby the balance of forces—of the electromotive forces created through cardiac activity.

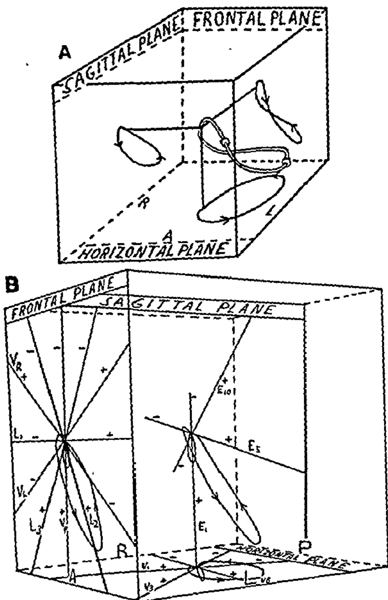


Fig 2 —Diagram of spatial cardiac vector loop as inscribed throughout cardiac cycle A, QRS loop, drawn as though located … tions of this loop onto the horizontal, sag… seen through the sides of a transparent … posed on the lead systems of derivation of … planes

of the three matched or simultaneously recorded electrocardiograms which formed the basis for the analysis. It has only historical interest now. Later, Mann (58, 59) developed a special three-pole galvanometer in a search for instrumental means for combining the three standard leads to one single curve.

Advances in design of cathode ray tubes with conventional deflection plate positions (right angles) were successfully applied by a number of investigators (73, 74, 83, 94), which obviated further search for specially designed instruments. Attempts have been made to record the spatial characteristics of the cardiac vector loop by projecting it to more planes than the frontal one alone (13, 19, 21, 46, 47, 65, 71, 83).

TECHNIC.—If the theoretic premise for treating the electric field generated by the heart as a dipole is correct, there is one spatial cardiac vector which contains all the information about the balance of forces generated and the sequence at which they are released. This cardiac vector can be recorded with absolute accuracy by any sensible technic advocated, provided one corrects or can correct for errors inherent within them and the biologic substrate subjected to the analysis—the body and the nonhomogeneous conducting properties of its tissues—within which the electromotive field exists.

ELECTRODE PLACEMENT

Since the cardiac vector has a three-dimensional extent, its recording requires it to be viewed as projections to two or more preselected planes or to be prepared for stereoptical viewing (40, 67, 71) This may be likened to an architect's lot: a perspective drawing will attract the eye and imagination of the prospective purchaser of a home The same sketch, although impressive to the eye and imagination, will prove useless to the contractor who requires exact dimensions. Drawings showing the house projected to planes with a simple geometric relationship to each other (at 90° angle) will serve his purposes, i e , a floor plan (transverse or horizontal plane), a side view (sagittal plane), and a front view (frontal plane projection), since they will contain exact dimensions.

There are several systems of electrode placement in use at present However, only two seem to be used by a majority of investigators: (*1*) the one based on an extension of the equilateral triangle of Einthoven, as proposed by Wilson *et al* (93) and applied by Conway *et al.* (14),

Information about the normal instantaneous progressive balance of forces is sufficient so that deviations from the normal range can be ascribed to specific changes of the contributing muscle elements. Abnormal sequences of stimulation can be identified with equal facility.

DEVELOPMENT OF CONCEPT.—*In 1913, Einthoven, Fahr, and de* Waart (23) described the determination of the "resultant manifest potential" based upon the scheme of the equilateral triangle, the vertices of which are represented by the right arm, left arm, and left leg electrodes. By this technic the net amplitude and direction of the QRS complex in each of the three standard extremity leads were plotted along the sides of the triangle. The magnitude and direction of the resultant manifest potential differences were then determined geometrically by the use of polar coordinates. The manifest potential difference thus obtained represented as a single vector the magnitude, direction, and sense of the total electromotive forces of the heart as *projected to the plane formed by the three leads.* The authors also determined the values, i.e., the angles and the magnitude of the manifest resultant electromotive force, for several instances during cardiac activation. Although they did not follow their computation through to the point of geometric presentation, their data were so complete that it could be done decades later (97). Einthoven *et al.* thus observed *that homogeneous peaks of the QRS complex in the three leads did not* occur in phase, i e., peaks of the R waves did not occur simultaneously Williams was the first to combine the synchronously occurring potentials of the extremity leads into a single curve which he termed the vectordiagram He concluded that the difference in phase of homogeneous peaks of the QRS complex depicted the vectorial character of the potential changes in the heart In a discussion of the spread of the excitation wave, Fahr, in 1920, gave an account of vector interpretation of the electrocardiogram with emphasis on the phase relationship of the potentials of the three extremity leads He also postulated that the criteria prevalent at that time for the diagnosis of right and left bundle branch block were incorrect and suggested criteria which are now considered correct.

*Whereas Williams plotted the vector diagram onto a polar coordi*nate system, Mann (58) adapted this technic of vectorial reconstruction to a rectangular coordinate system, determining the value of the horizontal axis from lead 1 and for the vertical axis geometrically from *leads 2 and 3* $[(L2 + L3)/\sqrt{3}]$ Little use *was made of the time-con*suming technic, however, since each case required careful construction

central terminal) and the latter's position is unknown unless specifically determined by complex methods, the angle of the sagittal unipolar lead varies considerably in normal individuals, and markedly and unpredictably in patients with abnormal hearts. The distance of the back electrode from the electrical center will remain unknown and will be found to vary greatly where specifically determined. These difficulties were the basis of the impression that the tetrahedral system showed greater sensitivity than the cube system in detecting right ventricular hypertrophy (3). These discrepancies are more correctly explained as due to the unreliability of the unipolar lead to project correctly the sagittal voltages of the cardiac vector.

ORTHOGONAL ARRANGEMENT.—Schellong *et al* (72), in 1937, were the first to suggest that the equilateral triangle for the recording of the

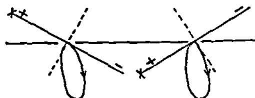


FIG 3.—Effect of positional change of an exploring electrode on the polarity of recorded voltage.

cardiac vector be abandoned. They placed the electrodes directly on the thorax and in an orthogonal arrangement. The center of the x, y, and z axes of the bipolar leads was left anteriorly and superiorly, thus close to the dipole center.

Duchosal and Sulzer (21) modified this technic but also used three bipolar leads, placing them most distant to the dipole center with their axes forming the adjoining three edges of an orthogonal body whose vertical axis had twice the length of the sagittal and horizontal axes (thus not a cube, as Schaffer *et al.* (70) and Goldberger (29) had misunderstood it to be).

CUBE ARRANGEMENT.—The unequal quantitative projection of the cardiac voltages unto the bipolar leads due to inequality of the internal angles in the orthogonal arrangement was corrected by the "cube" form of representation (Fig 4) developed at The Mount Sinai Hospital (36)

On the basis of exacting experiments, Gillard *et al* (28) concluded that the electrodes may be regarded as ideally placed when, (*1*) they

and (2) the orthogonal system of Schellong (71), its modification by Duchosal and Sulzer (21), and its subsequent modification to the cube system electrode placement (36).

In the technic used by Conway and co-workers, the equilateral triangle of Einthoven is considered as representing the frontal plane, and a unipolar electrode located somewhere on the back is considered the summit of an equilateral tetrahedron. The placement of the back electrode is such that it records the sagittal extent of the electromotive forces. Since the standard extremity and the unipolar extremity leads are components of the Einthoven triangle, the frontal plane projection of the vector loop recorded by this technic correlates exactly to conventionally obtained extremity leads However, it is not altogether valid to represent the forces recorded by the extremity bipolar leads in the form of an equilateral triangle nor to regard them as determining the frontal plane. The original proponents of the Einthoven triangle regarded it as an approximation only, Burger and van Milaan (9), den Boer (18), and Wilson and associates (91) have shown that the triangle determined by the three extremities is not equilateral because of marked discrepancy of the electric properties between source and electrode of the left arm and left leg, resistance and distance being the main factors responsible for this variability. It is also doubtful that these electrodes represent a plane parallel to the frontal plane. The plane, determined by the three electrodes, may deviate by as much as 35 degrees from the true frontal, clockwise or counterclockwise around a perpendicular axis (36) The effects of such a tilt may be negligible for the frontal plane projection, mostly resulting in a perspective distortion of the graph If used, however, as the basis of the more complex geometric system—the equilateral tetrahedron—the distortion may become too great for the graph to remain meaningful A further difficulty in the equilateral tetrahedron system is the location of the summit of the tetrahedron for the proper location of the unipolar lead V_B or back electrode A slight shift in the position of this electrode, up or down, or right or left, may result in complete reversal of the direction of rotation of the vector loop because of an altered position of the vector loop within the field of polarity of the lead (Fig 3). The incorporation of so-called unipolar leads into any system for the recording of the cardiac vector will make it particularly vulnerable to grossly inaccurate representations of the cardiac vector Since the direction of any unipolar lead depends upon the position of the exploring electrode in relation to the dipole center (as a result of producing a so-called

of forces affected the results in a quantitative but never qualitative way The electrodes are placed so as to represent four corners of a cube (Fig. 5), having the dipole center E at its center, and to be as equidistant from it as the anatomy of the thorax permits It should be noted that the cube is around the dipole center and not around the thorax, as interpreted by Schaffer *et al.* (70). Although the anatomic eccentricity of the heart influences indirectly the location of the dipole

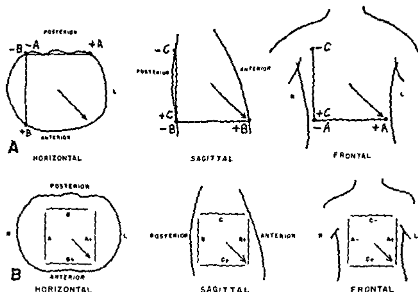


FIG 5 —Bipolar components of vectorcardiogram A, the bipolar components indicated on the outlined body. B, connections and polarity of the bipolar leads to the plates of cathode ray oscilloscopes

center, electrode placement is merely dependent on the eccentricity of the dipole center

The three bipolar leads are utilized, after suitable amplification, to reconstruct planes and, through connections to the plates of a cathode ray oscilloscope (Fig. 5), allow the instantaneous presentation of the cardiac vector as it appears projected to that plane. The electron beam of the cathode ray oscilloscope gives the resultant deviation of the cardiac voltages as they appear in two leads placed at right angles to each other, i e, the phase relationship of these voltages is obtained instrumentally.

are situated at the same distance from the heart, (2) the distance between each pair is the same, and (3) the distance between heart and electrode is about five times the heart's radius.

If one assumes the electric center of the heart to be at the center of a sphere, eight selected points on the surface of this sphere will form a cube, each point being equidistant from the center, E. The line from E to adjoining corners would determine equal internal angles The electrodes of three bipolar leads are applied to the thorax so as to form three adjoining edges of a cube (Fig 5). There are three bi-

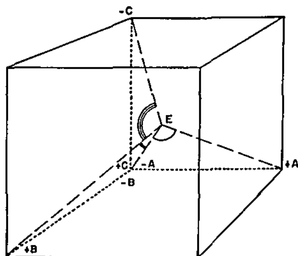


FIG 4—The internal angles of a cube being equal, the forces projected on bipolar leads *A*, *B*, and *C* are of comparable magnitude

polar leads A = horizontal, B = sagittal, and C = vertical They receive and record the cardiac voltages as projected to the interelectrode line Two such lines allow the construction of a plane since the angle is known (90 degrees). Thus, three planes can be reconstructed· (*1*) transverse or horizontal, (2) sagittal, and (3) vertical, commonly called frontal or parallel to frontal

The "cube" is constructed around the assumed position of the dipole center. For this purpose it is assumed to be located in the center of a sagittal plane passing just to the left of the sternum at the level of the fourth intercostal space Actual determinations of the mean dipole center showed this to be true in most normal and abnormal instances. The more significant deviations in severe pathologic balances

proposed by Duchosal and Sulzer (21), our cube system (35, 36), and a "corrected" vector system based on Cabrero Cosio's (11) principles—and found the cube system and the corrected "vectors" to be superior to the Duchosal-Sulzer orthogonal system

LEAD PLACEMENT.—Donzelot *et al.* (19) have advocated a lead placement in which V_7 represents the vertical component, V_2 the sagittal, and V_6 the horizontal. V_2 and V_6 are probably too close to the dipole center to reflect more than a distorted image of the cardiac vector. Furthermore, the two are at different levels. Although Jouve *et al.* correct for the latter, the proximity distortion of the components remains.

Lamb and Dimond (46) have recently advocated a reference system in which bipolar component leads are placed somewhat differently from any of the aforementioned orthogonal system In the absence of personal experience with it, I cannot comment on it.

Goldberger (29) in the most recent edition of his book proposes a combination of two different systems, Wilson's technic for the frontal plane and Donzelot's technic for the others No recorded graphs are shown. The configuration of vectorcardiograms based on such a hybrid system should be of interest. The vector drawings which accompany the text have the appearance of those previously published, recorded with more familiar but different reference systems

VALIDITY OF DIPOLE AND VECTOR CONCEPT

In the vector concept the electricity-generating heart is treated as a dipole source at the center of a sphere within an equivalent conducting medium. All electrocardiographic leads are regarded as recording the projection of the spatial cardiac vector onto the lines of derivation of the lead.

Several problems must be solved before the dipole theory can be accepted as a valid basis for vectorcardiography. The ideal physical situation requires a point source of electricity within a symmetrically shaped thorax of homogeneous conductivity

Duchosal and associates (20–22) gave one of the most important solutions to the problem They showed that unipolar leads recorded along the axis of diametrically opposed parts of the body gave electrocardiograms which were mirror images of each other in configuration and timing Jouve *et al* (44) confirmed these observations by animal

Accurate amplification free from interference can be readily obtained.* Presentation of the three planes may be accomplished in a triple oscilloscope arrangement, superior for research work, or successively *on a single cathode ray indicator* (*Technicon vectorscopes*) by means of a simple switch arrangement. A complete cardiac cycle is photographed. Timing and electronic technic for recording the rotation of inscription of the projected vector loop is accomplished by interrupting the beam 400 times per second (intensity modulation) and impressing an asymmetric beam intensity, shaping each of the "dashes" like an arrow (sawtooth pattern modulation) (35, 36, 87) (Fig. 6). The technic has been described in full detail elsewhere (35, 36). With the



FIG. 6—Teardrop or minnow shape of the time markers (400 cycles/sec) allows recording of the direction of inscription of QRS loop (clockwise)

instruments in our laboratory, the recording of the vectorcardiogram takes only 2 minutes. In most cases, with some experience, the results can be obtained simply by viewing the screen of the scope, without waiting for the permanent record When the Dumont Polaroid-Land camera is used, the permanent graph also becomes available a minute after its recording.

A camera is being developed which will permit automatic recording of the vectorcardiogram irrespective of rhythm, and the simultaneous recording of rhythm, electrocardiographically or vectorcardiographically, as first shown by Hollemann and Guckes (42)

Cabrero Cosio and Hernandez Aguilar (12) studied the electric field of the heart in cadavers with the aid of artificial dipoles They compared three orthogonal vectorcardiographic lead systems—the one

* We have been using the Technicon cardiograph machine for this purpose with great satisfaction

proposed by Duchosal and Sulzer (21), our cube system (35, 36), and a "corrected" vector system based on Cabrero Cosio's (11) principles —and found the cube system and the corrected "vectors" to be superior to the Duchosal-Sulzer orthogonal system.

LEAD PLACEMENT.—Donzelot *et al.* (19) have advocated a lead placement in which V_F represents the vertical component, V_2 the sagittal, and V_6 the horizontal. V_2 and V_6 are probably too close to the dipole center to reflect more than a distorted image of the cardiac vector. Furthermore, the two are at different levels. Although Jouve *et al* correct for the latter, the proximity distortion of the components remains

Lamb and Dimond (46) have recently advocated a reference system in which bipolar component leads are placed somewhat differently from any of the aforementioned orthogonal system In the absence of personal experience with it, I cannot comment on it.

Goldberger (29) in the most recent edition of his book proposes a combination of two different systems, Wilson's technic for the frontal plane and Donzelot's technic for the others. No recorded graphs are shown. The configuration of vectorcardiograms based on such a hybrid system should be of interest The vector drawings which accompany the text have the appearance of those previously published, recorded with more familiar but different reference systems

Validity of Dipole and Vector Concept

In the vector concept the electricity-generating heart is treated as a dipole source at the center of a sphere within an equivalent conducting medium All electrocardiographic leads are regarded as recording the projection of the spatial cardiac vector onto the lines of derivation of the lead.

Several problems must be solved before the dipole theory can be accepted as a valid basis for vectorcardiography The ideal physical situation requires a point source of electricity within a symmetrically shaped thorax of homogeneous conductivity

Duchosal and associates (20-22) gave one of the most important solutions to the problem They showed that unipolar leads recorded along the axis of diametrically opposed parts of the body gave electrocardiograms which were mirror images of each other in configuration and timing. Jouve *et al* (44) confirmed these observations by animal

experiments. It thus appeared that Einthoven's hypothesis, viewing the electricity generated by the heart as a single dipole, and which he had applied only to bipolar extremity leads, was equally valid for all leads, precordial and others. The voltage of any given electrocardiogram depended on the distance of the lead electrode from the dipole source and the resistivity of the intervening medium. It should be possible, therefore, to predict the exact configuration of electrocardiograms from the spatial vectorcardiogram. Such derivations show an astounding correlation, provided they are recorded from points of a plane which passes through the dipole center (Fig. 2).

Obviously, the intersection of two or more electrode axes furnishing sets of mirror image electrocardiograms should determine the spatial position of the dipole center rather well. This formed the basis of a convenient technic (35) for its localization which possibly made up through its simplicity for what it might be lacking in accuracy. When the potential of a given unipolar lead is connected to the plates of a cathode ray oscilloscope, that potential will be neutralized by a potential of reverse polarity but of identical magnitude. The two unipolar leads which neutralize each other lie on opposite sides of the electric center, E. The electric center (35, 36) rather than the anatomic center (21) was used for the cube placement of electrodes for vectorcardiography. Indirect evidence allows the postulate that the dipole center remains essentially stationary during cardiac activity This is probably due to a rather proportionate change of quantities contributing toward the balance of forces In instances of left bundle-branch block with its incoordinate sequence of excitation, a shift of the dipole center might be expected. Marked disturbances of forces, as they occur in myocardial infarction, may become similarly effective Exact knowledge of the behavior of the dipole center seems important for an understanding of the cardiac electric field This information can be obtained experimentally with electronic instruments, the construction of which, however, would involve considerable expense and labor. The effect of the anatomic displacement of the heart during its contraction is equally unknown

Recently, Schmitt and associates (77, 78, 54) presented further evidence, on the basis of electrocardiographic mirror pattern studies, for the validity of the dipole concept for electrocardiographic interpretation They found excellent cancellation in normal subjects and most patients studied They regarded the infrequently found poorer can-

cellation results to be due in large part to technical limitations; no evidence was seen that it is due to interference of local patterns.

As mentioned before, scalar electrocardiograms can be derived from the spatial vectorcardiogram with good correlation Duchosal *et al.* (21, 22) and the author with his co-workers (35, 38, 75, 76) were able to accomplish this. Duchosal and Grosgurin (20) thought the occasionally encountered differences were in some cases due to the possible simultaneous existence of two electric centers. This, however, seems to be an unnecessary assumption. When correlation of unipolar chest leads to the transverse, or horizontal plane projection are being made (8), such unipolar chest leads should be recorded from the periphery of a plane passing through the dipole center Corrections for angular or perspective projection should be taken into account when conventionally recorded chest leads are being used whose lead axis from the electrode location or "point" of exploration to the dipole center (null point, electric center) may form an angle of considerable magnitude. Recognition of the limitations of exact correlation for graphs recorded with technics based on different concepts will obviate critical conclusions that one or the other is inaccurate (8).

The ingenious construction of a panoramic vectorcardiograph by Milnor *et al* (60) allowed them to study the problem more directly and with exactness. Essentially through the use of sine-cosine potentiometers, instrumental transformation of rectangular coordinates to polar coordinates was achieved (45) Corresponding to the selected potentiometer positions, the spatial cardiac vector can be viewed from any angle desired (45) When this unit is combined with an adding circuit, scalar electrocardiograms can be calculated as the projection of the spatial vectorcardiogram on any single axis. By correcting for vertical deviations of unipolar chest leads from the horizontal plane at the level of the dipole center, they were able to record in fullest detail curves calculated from the spatial vectorcardiogram identical to actually recorded unipolar chest leads

Identical results were obtained in our laboratory with a simple and inexpensive apparatus (31) by adding the three component leads through an adding circuit (45) and determining the angle of derivation as the ratio of amplification or standardization of the component leads Treating component leads as lines, the ratio of their length (standardization) will determine the angle of the lead axis desired (arc tangent alpha) These can be applied to the transverse plane using

component lead A and B, injecting vertical correction (component C) as required in comparison to a directly recorded chest lead. This may be likened to trigonometry, accomplished electronically through variations of standardization of the components used and, again electronically, summating them (addition or subtraction). It seems possible to reconstruct all minute details of chest leads although the cube component leads have been obtained at as great a distance as the anatomy *of the thorax will allow (31).**

Thus, with added refinement in technic, it may be held as established that all leads recorded from the surface of the body can be regarded as derivations of the spatial cardiac vector. No longer should chest leads be considered inadequately remote, and therefore subject to local interference. It should be realized that the heart as a source of electricity can be treated as a dipole, that its electromotive field can be presented through the spatial cardiac vector, and that it contains all the information susceptible to instrumental analysis. All electrocardiographic leads are a projection of this vector onto the lead line of derivation (Fig. 2) All the information which can be obtained from them is contained within the cardiac vector or its recording, the spatial vectorcardiogram.

It should be emphasized that conventional electrocardiograms are devoid of phase relationship unless recorded simultaneously. This information, so readily available in the vectorcardiogram, can be plotted only with time-consuming effort. As a rule, it cannot be obtained by viewing the component leads even when they are recorded at higher than conventional speed

Spatial Vectorcardiogram

The representation of the vectorcardiogram of ventricular activation consists of a point of origin (white center) with a rapidly inscribed large loop (QRS loop), followed by a much more slowly inscribed smaller loop (T loop) which originates from the point of return of the QRS loop (Fig 6) Since the vectorcardiogram is recorded with a stationary film, the white center of the graph corresponds to the isoelectric line of the normal electrocardiogram, i.e., the fixed point at

* The instrument, which evolved from discussions with Dr A Helm, has proved most helpful in teaching the relationship of unipolar leads to spatial vectorcardiography.

which the electron beam of the cathode ray oscilloscope is photographed during the P-R, ST, and T-P intervals. Normally, QRS and T loops are almost completely closed, i e , their point of origin and their point of return are essentially identical. A dissociation of these will result in an open loop. In the scalar electrocardiogram, this is registered as a deviation of the ST segment. An ST vector, as it appears during ventricular activation, is indicated originating at the point of origin of the QRS loop and directed to the point of return.

Formerly, the direction of inscription had to be noted for each plane, but now is automatically recorded (Fig. 6).

The rate and speed of inscription can be determined by comparing the distance between the interrupted segments of the vector loop Care should be taken to corroborate alteration of speed from two planes, since transient, initial, or terminal slowing may be due to a perpendicular relationship of the vector loop to the plane of projection.

From the spatial cardiac vector as projected to three selected planes, any of the configurations of the routine scalar electrocardiograms can be derived, unipolar as well as bipolar (Fig 2). The justification for it and its limitations have already been discussed

DERIVATION OF SCALAR ELECTROCARDIOGRAPHIC LEADS

So-called unipolar leads were thought to record the potential under the exploring electrode without interference from the so-called indifferent electrode. This concept cannot be supported any longer. A "unipolar" lead system is connected on one side of the galvanometer with the electrode and on the other with the central terminal system proposed by Wilson Electrically, the dipole center and the central terminal are identical locations within the electromotive field of the heart Such a lead therefore records the voltages of the cardiac vector along a lead line from the point of exploration through the dipole center E (Fig. 7, *A*) (35, 38). It is also referred to as the axis of derivation. According to the adopted convention of polarity, whenever the loop is inscribed toward the electrode, an upward or positive deflection is recorded in the electrocardiographic record, whenever the loop is inscribed away from the exploring electrode beyond E, a negative deflection is recorded. The amplitude of the derived complex depends on the amplitude of the projected vector upon the lead line of derivation. Since all unipolar leads have the exploring electrode

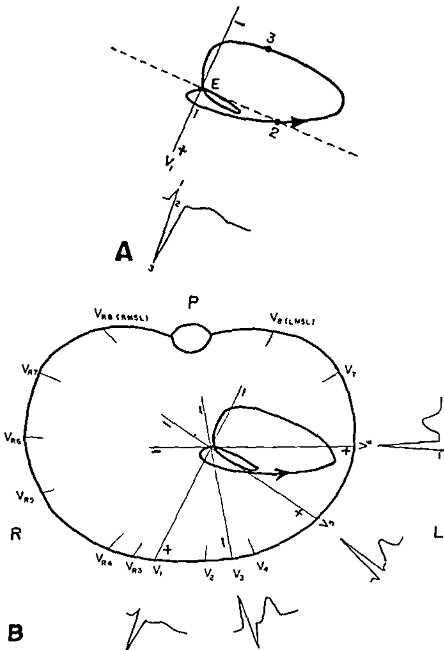


FIG. 7.—Relation of electrocardiographic leads to vectorcardiogram *A*, derivation of unipolar leads from vectorcardiogram, unipolar lead can be defined as recording the projection of the cardiac vector along a line of derivation drawn

paired with the dipole center as second pole, they have the unique ability to analyze the electromotive field in a radial fashion (35, 36), and they should therefore be regarded as the most exacting of scalar electrocardiographic technic (provided the Wilson central terminal with the enclosed resistors is used (35).

Bipolar leads record the potential difference between the two electrodes derived from the spatial cardiac vector through its projection upon the interelectrode line. The positive and negative field are divided through a perpendicular line upon the interelectrode line passing through the dipole center (35). In a bipolar lead the polarity of each electrode is arbitrarily defined, so that designation of a deflection as positive or negative depends on the polarity chosen.

Unipolar Chest Leads

The configuration of a unipolar chest lead recorded at the level of the dipole center E can be derived with great accuracy from the horizontal or transverse plane projection of the cardiac vector (Fig. 7, *B*) (35, 38, 76) Although the routine chest leads are recorded at different levels—and mostly below E—the discrepancies between the derived and actually recorded lead are generally insignificant They can be adequately corrected for by taking into account the distortion created by the angle of the actual line of derivation with the horizontal plane (vertical component correction) As discussed before, this can be accomplished electronically with astounding accuracy (31, 60).

Esophageal and Extremity Leads

Similarly, esophageal leads (Fig 7, *C*) can be derived from the sagittal plane projection, and from the frontal plane projection the bipolar and unipolar extremity leads (Fig. 7, *D*) (35, 38, 76) For the latter, the triaxial system of Bayley (6) combined with axes of the

---

from the point of exploration through the dipole center (E), with positive voltages recorded in the proximal field and negative voltages in the distal field Unipolar chest lead V_1 was chosen to make the diagrammatic example less abstract The dotted line, a vertical upon the line of derivation through the dipole center (E), separates the positive field (+) from the negative field (−) The numbers indicate corresponding points of the vector projection and of the scalar electrocardiogram *B*, horizontal plane projection of spatial vectorcardiogram and its relationship to unipolar chest leads Approximate position of chest electrodes is indicated by *R*, *L*, and *P* (patient's right and left sides and posterior aspect)

unipolar extremity leads was found to be most suitable. Since the plane of the Einthoven triangle is often found rotated along a vertical axis from a true frontal by as much as 35 degrees, lead 1 will not always be

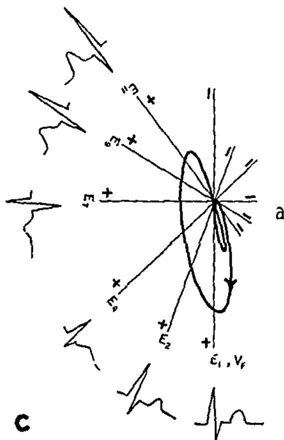


Fig 7, C—Relation of electrocardiographic leads to vectorcardiogram sagittal plane projection in relation to several esophageal leads, a, patient's anterior aspect The lowest esophageal lead, when placed in the stomach, has the same line of derivation as a unipolar lead V_F Relations of specific esophageal leads to the cardiac vector vary somewhat from patient to patient

exactly like component lead A Both should, under ideal conditions, be similar to a unipolar lead V_6, provided the angle of the line of derivation of V_6 from the horizontal plane affects its configuration only to a minor degree.

With a little practice, mere inspection of the plane projections of

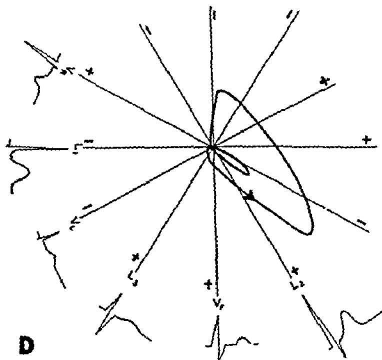


FIG 7, D—Relation of electrocardiographic leads to vectorcardiogram. relationship of horizontal plane projection to bipolar and unipolar extremity leads. *Bayley's triaxial system, to which are added the lines of derivation of unipolar* extremity leads, is superimposed on the frontal plane projection of the spatial vectorcardiogram

the spatial vectorcardiogram allows prediction of the findings of routine electrocardiograms

THE VECTORCARDIOGRAM

NORMAL

ADULTS —The normal spatial vectorcardiogram is oriented to the left, inferiorly, and somewhat posteriorly (Fig 8, A) Comparatively little variation is found, so far as the spatial position of the vectorcardiogram in adults is concerned (8, 35, 76) The normal QRS loop in the horizontal plane projection is characterized by an initial small deflection anteriorly and to the right The remainder of the loop is then inscribed in a counterclockwise direction to the left and somewhat

posteriorly. The initial deflection to the right inscribes a small R wave in V_1 and a small Q wave in V_6. The large deflection to the left inscribes a prominent S wave in V_1 and a prominent R wave in V_6

The normal QRS loop in the sagittal plane projection is characterized by an initial small deflection anteriorly and occasionally superiorly, the remainder of the loop being inscribed downward and somewhat

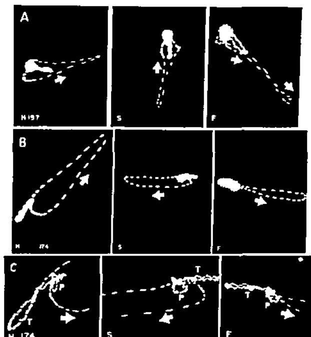


FIG 8 —Vectorcardiogram *A*, normal *B*, *C*, in left ventricular hypertrophy Note even spacing of time markers Tracing recorded at higher amplification (*C*) clearly shows the dissociation of the points of origin and return

posteriorly in a clockwise direction High esophageal leads are therefore essentially negative in configuration

In the frontal plane, the normal QRS loop is inscribed downward and to the left. There may be an initial small deflection to the right and/or superiorly. The more vertical loops in the frontal plane are inscribed in a clockwise direction, while the others are inscribed in a counterclockwise direction.

In the adult, the T loop is usually located anterior, inferior, and to the right of the QRS loop, by 10 to 30 degrees. Any marked increase in this angular deviation may prove to be an early vectorcardiographic

sign of myocardial damage, not readily available from scalar electrocardiograms.

CHILDREN.—The distinguishing characteristics of the normal routine electrocardiogram in children are mainly attributable to the more anterior orientation of the QRS loop (51) As in adults, the QRS loop in the horizontal plane is inscribed in a counterclockwise direction, but the loop is oriented more anteriorly and may extend an appreciable distance to the right. The precordial leads in children are thus characterized by R waves of increased amplitude and S waves of decreased amplitude over the right precordium.

The sagittal plane projection is characterized by an increased portion of the QRS loop lying anteriorly, as compared to the adult. The loop is inscribed in a clockwise direction, as in adults. At times, fairly large terminal portions of the QRS loop are inscribed superiorly and posteriorly.

In normal children, most of the frontal plane projections of the QRS loop are inscribed in a clockwise direction. Terminal portions may be found lying superiorly and to the right, so that prominent R waves are found in VR and prominent S waves in lead 1.

In the neonatal period (up to 2 months), the vector may be oriented toward the right, resulting in occasional clockwise rotation of the QRS loop in the transverse plane projection. In some infants at this age it may therefore be difficult to obtain decisive differentiation between the normal and right ventricular hypertrophy (24, 31, 70).

IN LEFT VENTRICULAR HYPERTROPHY AND LEFT BUNDLE-BRANCH BLOCK

The electrocardiographic distinction between left ventricular hypertrophy and left bundle-branch block is sometimes difficult when only conventional electrocardiograms are available However, the spatial vectorcardiograms display certain characteristics which permit the ready diagnosis of conduction delay (38, 66, 75, 99)

In the presence of left ventricular hypertrophy, the QRS loop in the horizontal projection is characterized by an initial deflection anteriorly and somewhat to the right The loop is then inscribed to the left and posteriorly in a counterclockwise direction The long axis of the QRS loop is more posterior than in normal persons, and there is no appreciable alteration in the distances between the time markings (Fig. 8, *B*). The sagittal plane projection of the QRS loop is inscribed

in a clockwise direction, and the loop is oriented more posteriorly than normally. The QRS loop in the frontal projection is oriented more to the left than in normal persons, and is usually inscribed in a counterclockwise direction The T loop in each projection lies opposite the QRS loop and there is no evidence of altered proximity of the time markings. The QRS loop may fail to close prior to the inscription of the T loop resulting in an S–T segment deviation in the routine electrocardiogram.

In the presence of left bundle-branch block, the time markings are

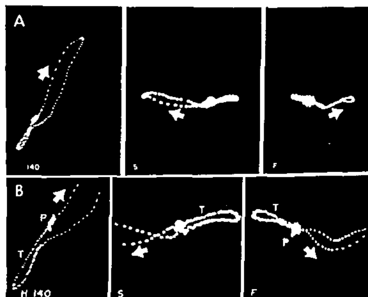


FIG 9 —Vectorcardiogram in left bundle-branch block *A*, slowed inscription of middle segment of loop *B*, tracing recorded at higher amplification clearly shows the dissociated points of origin and return

much closer together (73, 97) *This increased proximity is usually seen* in the middle and late portions of the QRS loop (Fig 9) The main portion of the QRS loop in the horizontal plane projection is inscribed in a clockwise direction, in contradistinction to the rotation found in normal individuals and in those with left ventricular hypertrophy The QRS loop is oriented posteriorly, to the left, and often superiorly In each projection, there is increased proximity of the time markings The T loop is usually oriented opposite the QRS loop, and the QRS loop fails to close prior to the inscription of the T loop

In both left *ventricular hypertrophy and left bundle-branch block*,

the T wave is inverted in the electrocardiogram whenever the ventricular complex is essentially upright, since the T and QRS loops are oppositely oriented in each plane

Many forms of conduction disturbances have been encountered (31, 35, 97), distinctly different from so-called left bundle-branch block. Some have been discussed before (35), others await further study and thought.

In Right Ventricular Hypertrophy and Right Bundle-Branch Block

Routine electrocardiography may fail to distinguish right ventricular hypertrophy from right bundle-branch block. The precordial lead patterns are at times similar, and RSR′ patterns may be present over the right precordium in both entities (4, 35, 38, 50, 52). The vectorcardiographic patterns of each are distinctive, however (35, 38, 50, 51). Atypical right bundle-branch block (Wilson type) is characterized by alterations which are usually confined to the terminal portion of the QRS loop (50) (Fig. 10, *B*). In each projection this terminal portion is increased in duration, is slow and irregular in contour, and is directed to the right and anteriorly. This portion of the QRS loop produces the slow, widened S wave of lead 1 and V_6 and the R′ or late R wave of V_1, and may co-exist with any normal or pathologic variety of the QRS loop

In right ventricular hypertrophy there is no evidence of a transient slowing of conduction (35, 38, 49, 52, 53, 86). The QRS loop is oriented to the right, anteriorly, and inferiorly, in mild degrees of right ventricular preponderance; right, anteriorly, and superiorly in more severe degrees, and right, posteriorly, and superiorly in most marked degrees. When the right ventricular preponderance is mild to moderate (Fig. 11), the horizontal projection is characterized by an initial small deflection directed to the right and anteriorly, followed by a large deflection to the left and somewhat posteriorly, then sharply to the right and anteriorly with a clockwise return to the point of origin. A unipolar electrocardiogram recorded over V_1 will reveal RSR′ patterns identical to those encountered in patients with atypical right bundle-branch block. With increasing degrees of right ventricular hypertrophy, prominent R waves are inscribed over the right precordium and essentially negative ventricular complexes over the left (43, 80).

The initial part of the vector loop is probably inscribed by septal activation alone It is directed to the right, anteriorly, and somewhat superiorly. The septal vector in right ventricular hypertrophy at times becomes directed toward the left, sometimes even markedly so (27, 35, 53). This will result in low R waves, isoelectric segments, or even Q waves in right-sided chest leads.

In mitral disease, the results of vectorcardiographic studies appear

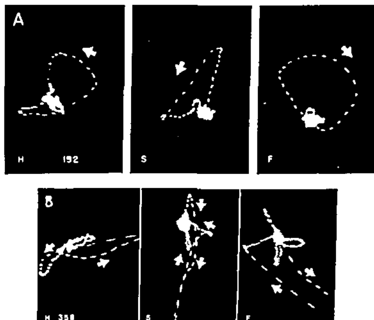


FIG 10—Vectorcardiograms in diaphragmatic infarction and right bundle-branch block *A*, diaphragmatic infarction caused superior displacement of QRS loop *B*, in right bundle-branch block the right anterior segment is slowly inscribed

inconsistent (25, 31) Despite severe right ventricular hypertension, the vectorcardiogram may reveal a normal balance. In other cases the correlation to right ventricular pressures resembles that encountered in congenital heart disease

The justification for the opinions of Wolff *et al.* (97) and of Sodi Pallares (79) that most right ventricular hypertrophy curves show partial or incomplete right bundle-branch block is questionable. It should be realized that there is no evidence for its existence in man. When the electrocardiogram is used as a basis for discussion, it should be recalled that similar patterns may have entirely different vectors

as their source. The results of animal experiments may require re-evaluation before their application to the far more complex pathology in man. There is only evidence that in so-called right bundle-branch block electric forces are being freed after most of the QRS loop has been inscribed. Their generation is usually slow and their vectors directed right anteriorly. They have the remarkable association of a T loop that most often is inscribed in the opposite direction. We know of no evidence to show that this force originates from the right ventricle

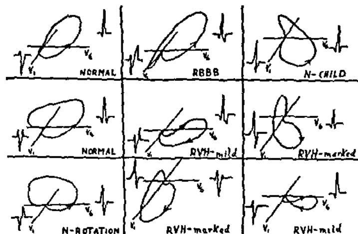


FIG 11.–Diagrammatic representation of horizontal projection vector loops, showing that dissimilar vector loops may result in similar electrocardiographic chest lead patterns. N, normal

and regard the available explanations with scepticism. It might even originate from an anterior segment of the left ventricle.

IN MYOCARDIAL INFARCTION

In the normal heart what is recorded is in essence the balance of forces generated by the opposing forces of the anterior and posterior walls, the diaphragmatic and superior aspects, and the right and left aspect of the heart When infarction of any part of the heart occurs, this area may be considered as electrophysiologically inert (5, 6, 35, 74, 82). As a result, this area does not contribute to the total resultant electromotive forces, and there is an apparent augmentation of the forces generated by the diametrically opposite area For example, an infarction localized to the anterior aspect of the heart will augment

the forces acting posteriorly, while an infarction of the diaphragmatic aspect will augment the forces acting superiorly. Since the QRS loop represents the total resultant electromotive forces of the heart, it will be oriented posteriorly when an anterior infarction occurs, and superiorly in a case of diaphragmatic infarction (Fig. 10, *A*). Q waves are registered in routine unipolar leads over the site of infarction since the forces are directed away from the infarcted area and therefore away from the electrode. R waves are usually registered opposite the site of the infarction since the forces are augmented in the area diametrically opposite the infarcted area. It is not intended to describe here the characteristics of the vectorcardiogram for each site of a myocardial infarction, but some indication of how a consideration of myocardial infarction on a vector basis can explain some otherwise confusing electrocardiographic patterns may be of value (35, 74).

Since prominent R waves are recorded opposite the site of infarction, the prominent R waves recorded in VR in extensive apical infarction need not be explained by rotational factors but by loss of apical electromotive forces. Prominent R waves in V_1 and V_2 can occur with posterolateral infarctions (35, 52, 74), while diaphragmatic infarctions may be accompanied by prominent R waves in supracardiac esophageal leads (35, 74).

There may be no evidence of infarction in routine electrocardiograms in some patients, but the vector loop may be displaced anteriorly (35, 74). In such instances, infarction of the posterior wall should be suspected.

It should be realized that with the vector concept the "window effect" as the origin of the Q waves is no longer tenable The Q wave was regarded as the reflection of the potential of the left ventricular cavity seen through a "window" within the left ventricular wall, i e, the electrically inert area of the myocardial infarction The shift of the vector loop may not be affected in the form of a localized marked deviation away from the exploring unipolar electrode, but affect it more widely and gradually. Although distinctly abnormal in the spatial vectorcardiogram, the electrocardiogram may fail to show evident abnormalities by not presenting diagnostic Q waves (35)

S-T Segment

Whenever the QRS loop fails to close prior to the inscription of the T loop, a deviation of the S-T segment is registered in the electro-

cardiogram. The direction and degree of this deviation may be determined by drawing a line from the dipole center E of the vectorcardiogram to the onset of the slowly inscribed T loop (35). This line represents the S-T vector, with the proximal end at E and the distal end as indicated. The orientation of this vector determines in which leads the S-T segment deviates. If this vector is directed to the right and superiorly, the S-T segment will be recorded as elevated in leads VR and V_1, and will be depressed in leads 1, VL, VF, and over the left precordium Although the S-T segment deviations in routine electrocardiograms have an essentially similar distribution in left ventricular hypertrophy, left bundle-branch block, and acute coronary insufficiency, the spatial orientation of the S-T vector differs distinctly in these three conditions (32, 35).

In acute myocardial infarction the S-T vector clearly points to the site of the current of injury, with the polarity as it appears during ventricular activation. The findings in instances of fibrinous pericarditis are similar, without associated changes of the QRS loop

T Loop

The electric expression of the process of repolarization is given by the T loop In certain lower species, such as the frog, the sequence of repolarization is similar to that of depolarization in that the first area depolarized is also the first to be repolarized The T loop, which is the result of repolarization, will therefore be oriented along the same axis but will be of opposite polarity, as compared to the QRS loop which is the result of depolarization. In the warm-blooded species, the spatial orientation of the QRS and T loops does not coincide exactly, so that there is a rather narrow range of angular deviation due to an altered time sequence of repolarization in relation to depolarization. Both processes differ, furthermore, in the speed with which the electromotive forces are generated The duration of the process of repolarization is not necessarily identical for each muscle area or layer (10, 35) Localized disturbances of the repolarization process may become evident by an altered contribution toward the T loop so that its spatial orientation is altered in relation to the long axis of the QRS loop Abnormal degrees of angular deviation of the QRS to T loop can be recognized before abnormal T waves are recorded in extremity or chest leads since a relatively large angular deviation is necessary before abnormal T waves are recorded (35). In cases of angina pectoris due to coronary

sclerosis, therefore, diagnostic vectorcardiograms yield more information than routine electrocardiograms.

Changes in the spatial orientation of T loops due to myocardial disease are distinctly different from those due to quinidine, emetine, stibophen (Fuadin), and hypopotassemia, moving anteriorly in the former and as a rule posteriorly in the latter (31).

Wolff-Parkinson-White Conduction

The characteristic element of aberrant atrioventricular and interventricular conduction (37), the delta wave, is shown most clearly in the spatial vectorcardiogram (33, 84, 97). Its absence probably precludes the diagnosis, since short P-R intervals have been encountered in cases of left bundle-branch block. The observation of Wolff and associates (97) that aberrant atrioventricular conduction can be found in association with so-called atypical right bundle-branch block has been confirmed (31) Despite investigation of a large number of patients without and with all varieties of heart disease, sufficiently diagnostic features have not been found as long as the abnormal conduction prevailed (31) Quinidine sulfate given intravenously in judicious doses has suppressed aberrant conduction in most instances in the author's experience This may become important in the diagnosis of congenital heart disease but should not be considered for cases of myocardial infarction.

P Loop

In the past, instrumental limitations made a satisfactory study of the P loop difficult. Greatly improved instruments have made it possible to record detailed curves Mitral disease, right-sided congenital cardiac defects, and pulmonary disease have shown rather characteristic P loops In left atrial enlargement they are triangular and oriented inferiorly in a sagittal plane, in right atrial enlargement they have a round contour, and are anteriorly and somewhat inferiorly oriented.

Atrial Flutter

Vector analysis of atrial complexes, the technic applied by Lewis (56), is in my opinion the most direct (and probably the only) method to prove or disprove the circus movement theory of Lewis as the sustaining mechanism of atrial flutter in human beings The application of

intracardiac and esophageal electrodes in human subjects with chronic atrial flutter and recording of the atrial vectors from the cathode ray oscilloscope by means of a high-speed motion picture camera (64 frames/second) revealed a continuously moving vector in several subjects so studied (39). It appears that Lewis' basic theory retains its validity. The experiments of Prinzmetal and associates (65) have not proved that circus movement does not exist. The lucid experiments of Rosenblueth and co-workers (68, 88), the investigations of Cabrera and Sodi Pallares (13), and our own work have greatly strengthened Lewis' theory. The widespread acceptance of experiments apparently refuting Lewis' theory is no indication that the basic tenet of the circus movement concept was understood in all its implications.

VECTORELECTROCARDIOGRAPHY

Grant and Estes (30) have proposed a simple method of applying vector concepts to the analysis and interpretation of the conventional electrocardiogram and have contributed greatly to familiarization with the vector concept. An attempt was made to establish the mean direction and magnitude of the QRS and T vectors. Since instrumental means for visualizing the cardiac vector are available, their technic serves no useful purpose. The use of conventional leads, when recorded at conventional speeds and not simultaneously, is a questionable practice since they lack phase relationship and detail which makes them unsuited for vector analysis.

VALUE OF VECTORCARDIOGRAPHY

The vector concept, with spatial vectorcardiography, has made a threefold contribution to electrocardiology. (*1*) It has provided the method of choice for the teaching of electrocardiography. (*2*) It has provided a superior diagnostic tool, at present as an adjuvant to clinical electrocardiography and containing the potentialities to replace the latter altogether (3) It has stimulated a new and intensive search for the answers to numerous related problems, old and new.

The vector concept is unitarian, treating all electrocardiographic leads as derivatives of the spatial cardiac vector through its projection upon the lead lines of derivation. Its basis is the dipole theory as first proposed in its elementary form by Einthoven The evidence presented in recent years in support of the dipole theory is most convincing

The unitarian character of the vector concept allows the presentation of all electrocardiographic leads to be related to each other as derivatives from the single spatial cardiac vector and makes the memorizing of patterns and configurations no longer necessary. The cardiac vector contains all the information about the balance of forces *at each instant. This balance is well established for the normal heart; it* shows great consistency in its basic characteristics, with few and minor variations. The considerably greater variability of the normal electrocardiogram is due to the fact that a normal spatial cardiac vector will produce differing projections through even minor changes of its spatial position in relation to the fixed lines of lead derivations. Normality is presented through the spatial cardiac vector in a simple way, whereas electrocardiographic recognition of a normal electrical behavior of the heart is more complicated. Thus, the numerous electrocardiographic facets of normality can be easily visualized as due to altered orientation of a normal cardiac vector to the fixed lead system Similarly, deviations from its normal characteristics can be ascribed to specific pathologic entities. The analysis of even the most complex set of electrocardiograms offers no difficulties when the spatial vectorcardiogram is available.

The determination of cardiac position and rotation from the electrocardiogram is not valid (35, 60). The configuration of a given lead depends not on the cardiac chamber facing it but on the projection of the cardiac vector to the lead line of derivation The relation of the spatial position of the cardiac vector to the cardiac position is too inconsistent to be significant Furthermore, more direct means are available to learn the position of a patient's heart

Similar caution should be applied to continued use of the intrinsic or "intrinsicoid" deflections (21, 35, 60). *At our present state of knowl-* edge, they cannot be regarded as signaling the arrival of the excitation wave under the exploring electrode but rather as representing the turning point of the cardiac vector in relation to the line of derivation *of the lead employed.*

The contributions of spatial vectorcardiography are not limited to the increased understanding and simplified teaching of electrocardiography (7). In our experience, it has become a most valuable diagnostic *tool, for the time being still to be treated as an adjuvant to* clinical electrocardiography although it is now routinely performed in the evaluation of patients for surgery for congenital as well as mitral heart

disease. Wolff and associates (97) have arrived at a similar conclusion on the basis of 800 cases studied with the cube reference system.

In the differential diagnosis of congenital cardiac defects, the spatial vectorcardiogram will clearly indicate the presence or absence of right ventricular preponderance whereas the electrocardiogram may leave great doubt or relay erroneous information This pertains not only to high R waves and RSR' patterns in right-sided chest leads but also to the peculiar rotation patterns not infrequently encountered in mitral heart disease (Fig 11).

In the differentiation of conduction disturbances, bundle-branch block, and left ventricular preponderance, it has proved an equally superior analytic tool Left bundle-branch block, left ventricular preponderance, and Wolff-Parkinson-White conduction are easily differentiated. Furthermore, a number of other conduction defects of entirely unsuspected nature can be found, the clarification of which requires further work and a good deal of critical thought. Those entities already known can be more easily detected and differentiated by the vectorcardiogram than by the conventional electrocardiogram

Myocardial infarction apparently can be diagnosed more often vectorcardiographically than electrocardiographically. The dependence in the latter technic on the appearance of Q waves is not necessary under the vector concept Therefore, abnormal orientation of the QRS and T loops appear sufficiently indicative for its detection. More ample clinical and pathologically confirmed material is still required to confirm and consolidate these impressions

ST and T vectors, although giving rather similar projections in scalar electrocardiograms, have dissimilar vectorcardiographic appearances As Bayley (5) had postulated correctly on theoretic grounds, the ST vector in acute myocardial infarction points to the site of the infarct Recorded cases show the disturbed balance due to the electrically inert infarcted area expressed in the QRS and T loop and its location indicated by the "pointing" ST vector with simple directness in a single graph

It should be fully appreciated that spatial vectorcardiography is a new tool Although the various investigators in the field have not agreed on a reference system, the cube system proposed by the author and his co-workers seems to have been successfully used in extensive clinical trials (24, 25, 27, 97), the results of which confirm and in part extend the findings originally reported by us

The ease with which vectorcardiograms can be viewed on the screen of a cathode oscilloscope, and recorded, will depend on the technical perfection of the equipment available to the investigator The apparatus used at The Mount Sinai Hospital for the past 5 years allows the viewing and recording of the spatial vectorcardiogram in about 2 minutes. A permanent record is always obtained, although the vectorcardiogram can be described and interpreted from the screen.

A simple technic for the uninterrupted automatic recording of vectors and the simultaneous recording of rhythmic events has been devised. Disturbances from respiratory movements of the thorax also can be eliminated by using a variation of an electric filter (34) previously designed for esophageal electrocardiography; it is required only rarely, however.

The use of cathode ray oscilloscopes will allow miniaturizing of amplifiers and possibly the use of transistor-powered amplifiers Technical difficulties in the design of a reliable and reasonably priced vector machine are not to be expected at the present state of electronic engineering.

Once suitable apparatus becomes available, it will be simpler and more logical to record the spatial cardiac vector itself, rather than to teach electrocardiography by means of the vector concept Until such time, the stimulus which this concept has supplied through renewed emphasis on old and new concepts will help to advance knowledge about the electric activity of the heart.

REFERENCES

1 Abildskov, J A, Burch, G E, and Cronvich, J A. The validity of the equilateral spatial reference system, Circulation 2 122, 1950.

2 Abildskov, J A, *et al* The spatial vectorcardiogram in right bundle branch block, Circulation 3 600, 1951

3 Allison, W D, *et al* Right ventricular hypertrophy A comparative study in spatial vectorcardiography with cube and tetrahedron coordinates, and quantitative spatial summation vectorcardiography, in *Program: 26th Scientific Session of American Heart Association, April 9–12, 1953* (New York American Heart Association, 1953)

4 Barker, J M, and Valencia, R The precordial electrocardiogram in incomplete right bundle-branch block, Am Heart J 38 376, 1949.

5 Bayley, R H An interpretation of the injury and the ischemic effects of myocardial infarction in accordance with the laws which determine the flow of electric currents in homogeneous volume conductors and in accordance with relevant pathologic changes, Am Heart J 24 514, 1942.

6 Bayley, R. H : On certain applications of modern electrocardiographic theory to the interpretation of electrocardiograms which indicate myocardial disease, Am Heart J 26 769, 1943
7. Burch, G E Vectorcardiography, A M A Arch Int. Med 90 137, 1952 (editorial).
8 Burch, G E, Abildskov, J. A, and Cronvich, J. A.. Studies of the spatial vectorcardiogram in normal man, Circulation 7 558, 1953
9 Burger, H. C, and van Milaan, J B.: Heart-vector and leads, Brit Heart J 8 157, 1946, 9.154, 1947, 10 229, 1948
10 Cabrera Cosio, E *Bases Eléctrophysiologiques de l'Eléctrocardiographie* (Paris: Masson & Cie, 1948).
11 Cabrera Cosio, E Le problème des dérivations bipolaires appliqué à la vectocardiographie, Acta cardiol 4 231, 1949
12 Cabrera Cosio, E., and Hernandez Aguilar, E. Campo electrico del corazon, Arch Inst. cardiol México 22 121, 1952
13 Cabrera Cosio, E, and Sodi Pallares, D.· Discusión del movimiento circular y prueba directa de su existencia en el flutter auricular clinico, Arch Inst cardiol. México 17.850, 1947
14 Conway, J P., Cronvich, J A, and Burch, G E : Observations on the spatial vectorcardiogram in man, Am Heart J 38 537, 1949.
15 Craib, W H A study of the electrical field surrounding active heart muscle [with an appendix by R Canfield], Heart 14 71, 1927
16 Craib, W H: *The Electrocardiogram*, Medical Research Council, Special Report Series no 147 (London 1930)
17. Cronvich, J A, Conway, J P, and Burch, G E Standardizing factors in electrocardiography, Circulation 2 111, 1950.
18 den Boer, W *Vectorcardiographie* (Utrecht thesis, 1951)
19 Donzelot, E, Milovanovich, J B, and Kaufmann, H *Etudes Pratiques de Vectographie* (Paris l'Expansion Scientifique Française, 1950)
20 Duchosal, P W, and Grosgurin, J R The spatial vectorcardiogram obtained by use of a trihedron and its scalar comparisons, Circulation 5 237, 1952
21. Duchosal, P W, and Sulzer, R *La Vectocardiographie* (Basle· S Karger, 1949)
22 Duchosal, P W, Grosgurin, J, and Sulzer, R Etude des relations entre le vectocardiogramme et les dérivations standard, unipolaires des membres et precordiales, Acta cardiol 3 273, 1948
23 Einthoven, W, Fahr, G, and de Waart, A Über die Richtung and die manifeste Grosse der Potentialschwankungen im menschlichen Herzen and ueber den Einfluss der Herzlage auf die Form des Elektrokardiogramms, Pflueger's Arch ges Physiol 150 275, 1913
24 Elek, S R, Allenstein, B J, and Griffith, G C The infant vectorcardiogram, in *Program 26th Scientific Session of American Heart Association, April 9–12, 1953* (New York American Heart Association, 1953)
25 Elek, S R, *et al* A correlation of the spatial vectorcardiogram with right ventricular hypertrophy, in *Program 26th Scientific Session of American Heart Association, April 9–12, 1953* (New York American Heart Association, 1953).

26 Fahr, G.: An analysis of the spread of the excitation wave in the human ventricle, Arch. Int. Med. 25.146, 1920.
27. Fowler, N. O, and Helm, R. A: The spatial QRS loop in right ventricular hypertrophy with special reference to the initial component, Circulation, 7 573, 1953
28. Gillard, G, Hendricks, J, and Taccardi, B.: Contribution à l'étude des bases physiques de la vectocardiographie clinique Act cardiol 6:868, 1951
29. Goldberger, E.: *Unipolar Lead Electrocardiography and Vectorcardiography* (3d ed; Philadelphia: Lea & Febiger, 1953).
30. Grant, R. P, and Estes, E. H, Jr: *Spatial Vector Electrocardiography* (Philadelphia: Blakiston Company, 1951).
31. Grishman, A: Unpublished data
32. Grishman, A., and Donoso, E. Vectorcardiographic analysis of the ST segment, in *Program 26th Scientific Session of American Heart Association, April 9–12, 1953* (New York· American Heart Association, 1953)
33. Grishman, A, and Jaffe, H L· Spatial vectorcardiography: Wide QRS complexes with short PR interval (the Wolff-Parkinson-White syndrome), J Mt Sinai Hosp 18 200, 1951.
34. Grishman, A, and Pelavin, M.: An electrical filter for esophageal electrocardiography for the attenuation of extraneous low-frequency potentials, J Mt. Sinai Hosp. 18 134, 1951.
35 Grishman, A, and Scherlis, L. *Spatial Vectorcardiography* (Philadelphia· W. B *Saunders Company, 1952*)
36. Grishman, A, Borun, E R, and Jaffe, H. L· Spatial vectorcardiography Technique for the simultaneous recording of the frontal, sagittal and horizontal projections I, Am Heart J 41 483, 1951
37 Grishman, A, Kroop, I G, and Steinberg, M F. The course of the excitation wave in patients with electrocardiograms showing short P-R intervals and wide QRS complexes (Wolff-Parkinson-White syndrome), Am Heart J 40 554, 1950
38 Grishman, A, Scherlis, L, and Lasser, R. P. Spatial vectorcardiography, Am. J Med 14 184, 1953
39 Grishman, A., *et al* Application of intracardiac, esophageal electrocardiography and vectorcardiography to the problem of the circus movement in man, Am J Med 8 395, 1950.
40 Guytan, A C, and Crowell, J W A stereovectorcardiograph, J Lab & Clin Med 40 726, 1952
41. Harris, S A The spread of excitation in turtle, dog, cat and monkey ventricles, Am J Physiol 134 319, 1941
42 Hollemann, W, and Guckes, E Das Triogramm und seine klinische Bedeutung, *Arch Kreislaufforsch 4 69, 1939*
43 Johnson, J B, *et al* The relation between electrocardiographic evidence of right ventricular hypertrophy and pulmonary arterial pressure in patients with chronic pulmonary disease, Circulation 1 536, 1950
44 Jouve, A, *et al* *La Vectocardiographie en Clinique* (Paris Masson & Cie, 1950)
45 Korn, G. A, and Korn, T M *Electronic Analog Computers* (New York McGraw-Hill Book Co, Inc, 1952)

46 Lamb, L. E., and Dimond, E. G.: A method for recording spatial vectorcardiograms, Am. Heart J. 44:165, 1952
47. Lamb, L. E., and Dimond, E. G. The spatial vectorcardiogram during the first decade of life, Am. Heart J. 44:174, 1952.
48 Langner, P. H. High fidelity electrocardiography. Further studies including the comparative performance of four different electrocardiographs, Am. Heart J. 45:683, 1953
49 Lasser, R. P., and Grishman, A. Vectorcardiograms obtained in patients with right ventricular hypertrophy whose electrocardiograms display an unusual axis deviation or left axis deviation IV, Am. Heart J. 41:901, 1951.
50 Lasser, R. P., and Grishman, A. Spatial vectorcardiography: VIII Right bundle branch block, Am. Heart J. 42:513, 1951.
51 Lasser, R. P., and Grishman, A. Spatial vectorcardiography in children: An analysis of high R-waves in right-sided chest leads, J. Pediat. 39:51, 1951
52 Lasser, R. P., Borun, E. R., and Grishman, A. A vectorcardiographic analysis of the RSR' complex of the unipolar chest lead electrocardiogram III, Am Heart J. 41:667, 1951
53 Lasser, R. P., Borun, E. R., and Grishman, A. Spatial vectorcardiography Right ventricular hypertrophy as seen in congenital heart disease: VII, Am Heart J. 42:370, 1951
54 Levine, R. B., Schmitt, O. H., and Simonson, E. Electrocardiographic mirror pattern studies II, Am. Heart J. 45:500, 1953
55 Levy, L., II, et al. Prominent R-wave and shallow S-wave in lead V_1 as a result of lateral myocardial infarction, Am. Heart J. 40:447, 1950
56 Lewis, T. *The Mechanism and Graphic Registration of the Heart Beat* (3d ed., London Shaw & Sons, Ltd., 1925)
57 Lewis, T., Meakins, J., and White, P. D. The excitatory process in the dog's heart I The auricles, Phil. Tr. Roy. Soc. 205B:375, 1914
58 Mann, H. A method of analyzing the electrocardiogram, Arch. Int. Med 25:283, 1920
59 Mann, H. The monocardiograph, Am. Heart J. 15:681, 1938
60 Milnor, W. R., Talbot, S. A., and Newman, E. V. A study of the relationship between unipolar leads and spatial vectorcardiograms, using the panoramic vectorcardiograph, Circulation 7:545, 1953
61 Myers, G. B., Klein, H. A., and Hiratzka, T. Correlation of electrocardiographic and pathologic findings in large anterolateral infarcts, Am. Heart J. 36:838, 1948
62 Myers, G. B., Klein, H. A., and Hiratzka, T. Correlation of electrocardiographic and pathologic findings in posterior infarction, Am. Heart J. 38:547, 1949
63 Myers, G. B., Klein, H. A., and Stofer, B. E. The electrocardiographic diagnosis of right ventricular hypertrophy, Am. Heart J. 35:1, 1948
64 Pantridge, J. F., et al. A study of the spatial vectorcardiogram in left bundle branch block, Circulation 1:893, 1950
65 Prinzmetal, M., et al. *The Auricular Arrhythmias* (Springfield, Ill. Charles C Thomas, Publisher, 1952)
66 Reid, W. D., and Caldwell, S. H. Research in electrocardiography, Ann. Int. Med. 7:369, 1933.

67. Rochet, J., and Vastesaeger, M. M Relief vrai et faux en stéréovectocardiographie, *Compt. rend. Soc. biol. 138:992, 1944*
68. Rosenblueth, A., and Garcia Ramos, J Studies on flutter and fibrillation II The influence of artificial obstacles on experimental auricular flutter, Am Heart J. 33 677, 1947.
69. Schaefer, H.: *Das Elektrokardiogramm* (Berlin Springer Verlag, 1951)
70 Schaffer, A. I, *et al* · Eccentricity as a cause for the difference between the vectorcardiograms registered by the cube and tetrahedral systems, Am Heart *J. 45.447, 1953*
71 Schellong, F: Grundzuge einer klinischen Vektordiagraphie des Herzens, Ergebn inn Med. u Kinderh 56 657, 1939 [Also issued as separate monograph (Berlin Springer Verlag, 1939)]
72. Schellong, F., Heller, S, and Schwingel, E: Das Vektordiagramm, eine Untersuchungsmethode des Herzens, Ztschr Kreislaufforsch. 29 497, 596, 1937
73 Scherlis, L, and Grishman, A. Spatial vectorcardiography· Left bundle branch block and left ventricular hypertrophy, II, Am Heart J, 41 494, 1951.
74 Scherlis, L, and Grishman, A Spatial vectorcardiography: Myocardial infarction, V, Am Heart J 42 24, 1951
75 Scherlis, L, and Grishman, A The correlation of spatial vectorcardiography with intracardiac and esophageal leads, J Mt. Sinai Hosp. 18 149, 1951.
76 Scherlis, L, Lasser, R P, and Grishman, A. Spatial vectorcardiography. The normal vectorcardiogram, VI, Am Heart J 42 235, 1951
77. Schmitt, O H, Levine, R B, and Simonson, E Electrocardiographic mirror pattern studies, I, Am Heart J 45 416, 1953
78 Simonson, E, *et al.*. Electrocardiographic mirror pattern studies. III, Am Heart J 45 655, 1953
79 Sodi Pallares, D Review of *Spatial Vectorcardiography* by A Grishman and L Scherlis, Am Heart J 44 322, 1952
80 Sokolow, M, and Edgar, A L A study of the V leads in congenital heart disease, Am Heart J 40 232, 1950
81 Sulzer, R, and Duchosal, P W Planographie Nouvelle méthode d'enregistrement électrocardiographique selon deux dérivations simultanées, Arch d mal du coeur 38 682, 1938
82 Vastesaeger, M M Quelques aspects vectocardiographiques de l'infarctus du myocarde, Acta cardiol 4 22, 1949
83 *Vastesaeger, M M, and Rochet, J La stereovectocardiographie et la stereo-* vectocardioscopie, méthodes cliniques d'étude de la répartition spatiale des potenti[illegible] 1944
84 Vastesa[illegible] Etude
85 Waller, A D A demonstration on man of the electromotive changes accompanying the heart's beat, J Physiol 8 229, 1887
86 Wenger, R, and Wick, E Vektorkardiographische Untersuchungen bei angeborenen Anomalien des Herzens und der grossen Gefasse, Cardiologia 20 1, 1952
87 Whipple, G H A simple technique for registering the direction of rotation of vectorcardiographic loops, *Am Heart J 44 384, 1952.*

88 Wiener, N , and Rosenblueth, A . The mathematical formulation of the problem of conduction of impulses in a network of connected excitable elements, specifically in cardiac muscle, Arch Inst. cardiol México. 16 205, 1946

89 Williams, H B . On the cause of the phase difference frequently observed between homonymous peaks of the electrocardiogram, Am J Physiol 35.292, 1914

90 Wilson, F N , and Johnston, F D The vectorcardiogram, Am Heart J. 16 14, 1938

91 Wilson, F. N , Bryant, J. M , and Johnston, F D On the possibility of constructing an Einthoven triangle for a given subject, Am Heart J 37 493, 1949.

92 Wilson, F. N ; Johnston, F D , and Hill, I G W.. The form of the electrocardiogram in experimental myocardial infarction IV. Additional observations on the later effects produced by ligation of the anterior descending branch of the left coronary artery, Am Heart J. 10 1025, 1935

93 Wilson, F N , Johnston, F D , and Kossmann, D. E. The substitution of a tetrahedron for the Einthoven triangle, Am Heart J 33 594, 1947

94. Wilson, F N , Rosenbaum, F F , and Johnston, F D Interpretation of the Ventricular Complex of the Electrocardiogram, in Dock, W , and Snapper, [illegible] s, Inc ,

95 [illegible] variations of a single electrode, Am Heart J 9 447, 1934

96. Wilson, F N , *et al* The precordial electrocardiogram, Am Heart J 27 19, 1944

97 Wolff, L , Richman, J L , and Soffe, A M Spatial vectorcardiography. Review and critique, New England J Med 248 810, 851, 1953

The L.E. Cell Phenomenon

MALCOLM M. HARGRAVES

Division of Medicine, Mayo Clinic, Rochester, Minn

SYSTEMIC LUPUS ERYTHEMATOSUS, because of its accompanying skin manifestations, was originally considered primarily of interest to dermatologists New diagnostic procedures, however, have disclosed that dermatologic manifestations may be but one of the protean expressions of this disease With recognition of the fact that systemic lupus erythematosus is responsible for many heretofore mysterious fevers of indeterminate origin, as well as for obscure cardiovascular-renal, pulmonary, gastrointestinal, osseous, and central nervous system disorders, it has become necessary to think of the disease as a differential diagnostic possibility in every field of medical practice The L.E cell phenomenon, and its utilization as a diagnostic laboratory procedure, has contributed its part in this better recognition of a highly malignant disease process It is to be hoped, however, that the phenomenon will contribute even more as a clinical investigative tool.

In June, 1953, an exhibit prepared for the Annual Session of the American Medical Association, in New York, showed that interest centered mainly in interpretation, methods, reliability, and specificity of the L.E cell phenomenon when used as a laboratory aid to clinical diagnosis Here, therefore, an attempt will be made to present, simply and pictorially, the basic observations, interpretations and concepts of the phenomenon for those engaged in clinical medicine Since the L.E cell phenomenon is a contribution of morphologic hematology to medicine, free use has been made of photomicrographs of blood and bone marrow preparations These photomicrographs help to distinguish the L.E cell phenomenon from nucleophagocytosis, with which it may be confused

DEFINITION

In the blood and other tissues of individuals afflicted with systemic lupus erythematosus is a unique substance commonly referred to as the L.E factor. Under certain conditions *in vitro*, the L.E. factor, which may or may not be active *in vivo*, can cause nuclear disintegration of body cells, particularly blood cells. I prefer to call this the L.E. cell factor, feeling that it is an activated form of the inactive L.E. factor.

In previous publications, the L.E. cell factor has been referred to as a lytic agent, since it causes the chromatin of cell nuclei to lose its structural pattern and become a relatively homogeneous, swollen mass which ruptures the nuclear membrane. This phenomenon is referred to as nucleolysis, or chromatolysis. It has been shown to be due to depolymerization of the desoxyribose nucleic acid of cell nuclei.

It must be emphasized that this biochemical nucleolytic action of the L.E. cell factor is the fundamental observable reaction involved in the L.E cell phenomenon. Failure to understand and recognize this morphologic change renders an observer incapable of interpreting the results of laboratory work in which the L.E cell phenomenon is employed as a diagnostic procedure.

Associated with this nucleolysis, the involved cell usually ruptures, leaving a free, lysed nuclear mass (called "globs" by Lee), which now becomes a foreign body. This lysed nuclear mass is chemotactic and attracts phagocytic cells. Neutrophilic polymorphonuclear leukocytes usually are the phagocytic cells attracted, although others may take part in the phenomenon Under proper conditions, these cells can be seen to migrate and to agglutinate about this lysed nuclear material Such a mass of phagocytic cells about a lysed nuclear mass is commonly referred to as a rosette In both vital and fixed preparations the lysed nuclear mass can be seen to be engulfed by the phagocytic cells In the living state, once this material has been engulfed by a phagocyte, the other cells which have been attracted to it are seen to move off in other directions, indicating loss of chemotaxis The resulting cell, which now contains a lysed nuclear mass within a digestive vacuole of its cytoplasm, becomes an L E cell.

Each aspect of the L E cell phenomenon will be discussed in detail in the following sections, and in the final section the application of the L E. cell phenomenon to clinical medicine, in the form of laboratory procedures.



FIG. 1 (*above*) —Typical L E cell with associated bone marrow elements, i e, normoblast, eosinophil, lymphocytic nucleus, and two neutrophils This L E. cell is a bilobed, neutrophilic, polymorphonuclear leukocyte, and its normal appearing nucleus is pushed somewhat toward the periphery of the cell by the inclusion bod [illegible]
nuclear ma [illegible]
and is a re [illegible]

FIG. 2 [illegible]
one lobe is undergoing lytic change (depolymerization of desoxyribose nucleic acid) Note that the affected lobe is greatly swollen, its chromatin pattern is disappearing, its staining reaction is less dark, and it is beginning to look smooth and homogeneous The second lobe is still relatively normal in appearance, although crowded to the periphery of the cell

L.E. CELL PHENOMENON AND PATHOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus is of unknown etiology; in fact, it is even of disputed pathologic definition. On what grounds, then, is one to accept a diagnosis of systemic lupus erythematosus and what is the relation of the L.E. factor to the accepted pathologic lesions? The answer to this question is basic in determining the specificity and clinical value of the L.E. cell phenomenon. Klemperer (26), one of the foremost students of the disease, and Lee, Michael, and Vural (32), all working at the Mount Sinai Hospital, New York, recently correlated the L.E. cell and histopathologic findings of material from their own laboratories. Klemperer emphasized that fibrinoid degeneration is a feature of many diseases ("collagen diseases") while not in every case of clinical systemic lupus erythematosus are the "pathognomonic cardiac, renal or vascular lesions" found. On the other hand, he has found the "hematoxylin-staining bodies" a constant histologic characteristic, occurring only in the tissues of victims of systemic lupus erythematosus. He and his associates reviewed material which he had on hand and found hematoxylin-staining bodies to be present in 32 of 35 instances. Since then, 12 more consecutive examinations have yielded an incidence of 100 per cent of these characteristic tissue lesions, Klemperer noted that they can be found to some extent in most organs if careful search is made. Control material has not revealed similar lesions and Klemperer expressed the belief that "it can now be concluded that presence of the hematoxylin-stained bodies is the pathognomonic criterion for the postmortem diagnosis of systemic lupus erythematosus."

Cytochemical studies have shown that the hematoxylin-stained bodies contain depolymerized desoxyribose nucleic acid. Lee, Michael, and Vural have shown, by the photometric methods used in study of hematoxylin-stained bodies, that the inclusion body of the L.E. cell also contains depolymerized desoxyribose nucleic acid. It was concluded that the lysed nuclear mass forming the L.E. cell inclusion body was optically and chemically identical with the hematoxylin-stained body. Klemperer further stated that the finding of the L.E. cell in blood and bone marrow preparations from patients suffering from systemic lupus erythematosus has been confirmed "by an ever increasing number of investigators, and the specificity of the lupus erythematosus cell. . .

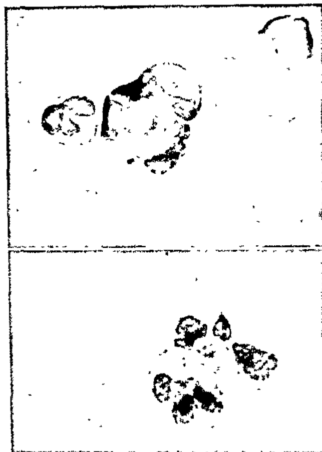


Fig 3 (*above*) — An interesting demonstration of chromatolysis One cell (a bilobed neutrophil) is an L E cell with rounded, lysed nuclear mass within its cytoplasm Note that a lobe of the phagocytic neutrophil overlies this inclusion body and has in turn undergone chromatolysis In this smooth, homogeneous, lysed lobe the progression of the lytic process comes to a rather sharp line of demarcation, with normal chromatin pattern beyond The same process may be seen in the adjoining nucleus

Fig 4 (*below*) — Four neutrophils clustered around a lysed nuclear mass Note that some chromatin pattern is still evident in this mass and that chromatolysis is in the process of breaking up the nuclear pattern This rosette demonstrates the chemotactic effect of the lysed nuclear mass, which now becomes a foreign body with the phagocytic cells being attracted to it Under supravital conditions, the cells surrounding this mass can be seen to engulf the material and become L E cells

has been fully established. Thus a valid criterion has been found for the diagnosis of systemic lupus erythematosus in the living."

Klemperer also has expressed the belief that the hyaline thrombi within glomerular capillaries seen in association with wire looping, and the fibrinoid material in the walls of larger vessels, are deposits of material "derived from a progressive breakdown of nucleoproteins and represents histone and other protein residues which impregnate the vessel wall. Since the process of DNA [desoxyribose nucleic acid] depolymerization is specific for systemic lupus erythematosus, it is justified to believe that the end product found impregnating the vessel wall is also characteristic. In other words, it is suggestive that the fibrinoid in lupus specifically consists of the breakdown products of nucleoproteins, while fibrinoid occurring in other diseases is a different protein."

L.E. FACTOR (L.E. CELL FACTOR)

If Klemperer's view that the fundamental lesion of systemic lupus erythematosus is depolymerization of desoxyribose nucleic acid is to be accepted, some noxious agent or predisposing set of circumstances still is responsible for the specific biochemical reaction which produces the specific pathologic lesion At present this noxious agent is known as the L.E. factor.

✓ The L E. factor probably occurs in all of the tissues and fluids of the body, although investigators are best acquainted with it in the blood Observation, however, has not established that this L.E factor is active *in vivo*, although it may be assumed that clinical manifestations of systemic lupus erythematosus are expressions of the activity of the L.E factor With one exception (9), I know of no instance in which blood, bone marrow, or tissue has produced L E. cells without a preliminary period outside the body, i.e, in all but one instance the L E cell was produced *in vitro* The phenomenon is not seen in "direct" blood and bone marrow smears from victims of systemic lupus erythematosus, whereas indirect preparations from the same material yield positive results The argument might be carried further, that is, it might be said that all necropsy material has had a varying period *"in vitro"* after the victim's death and until biochemical activity is stopped by tissue fixation. The same might be said about blister fluid, pleural and peritoneal exudates, and other fluids which have yielded L E. cells or hematoxylin-staining bodies In study of fixed bone marrow tissue, the L E. cells are almost invariably found (serial sections) at the



Fig 5 (*above*) —Phagocytosis of a lysed nuclear mass by 2 neutrophilic, polymorphonuclear leukocytes Part of the mass has been taken into the cytoplasmic vacuole of each of the cells and the mass is constricted by the efforts of the cells to ingest it Another neutrophil has been attracted to the mass but appears unsuccessful in its phagocytic attempt

Fig. 6 (*below*) —Phagocytosis of a mitotic figure in which the individual chromosomes have undergone chromatolysis The engulfing cell is a neutrophilic metamyelocyte and its cytoplasmic pseudopods can be seen advancing along the sides of the mass

periphery of the bone marrow particles. In a recent study of a section of spleen obtained by biopsy from a patient with systemic lupus erythematosus as diagnosed on the basis of the finding of L.E. cells by the clotted blood technic, the nuclei of the cells for a depth of 3 or 4 cell layers had all lost their chromatin pattern; homogenization and swelling were evident. Cells deeper in the splenic tissue contained normally stained nuclei. Was the change in the superficial cells equivalent to an *in vitro* reaction?

On the other hand, if it is assumed that the L.E. factor is active *in vivo* and is not merely a by-product of the disease complex, then *in vitro* conditions markedly increase the ability of the L.E. factor to initiate the L.E. cell phenomenon. For this reason, I prefer to speak of the "L.E. cell factor" at this point, believing that the L.E. factor of the *in vivo* state becomes activated to the L.E. cell factor in the *in vitro* state.

L.E. CELL FACTOR

The L.E. cell factor exists in the plasma and serum of individuals suffering from systemic lupus erythematosus. This factor, as was said when the L.E. cell phenomenon was originally described, has the unique ability to initiate the L.E. cell phenomenon by causing lysis of the nuclei of body cells, particularly of blood cells. The factor is relatively stable and maintains its potency for long periods if kept frozen, although boiling destroys its activity. The L.E. cell factor has been shown to exist in the gamma globulin fraction of the plasma proteins, as separated by electrophoresis. This factor is antigenic and, when injected into rabbits, will produce an antibody which in turn suppresses the L.E. cell phenomenon. The factor can induce the L.E. cell phenomenon when mixed with normal human blood or bone marrow cells, as well as when mixed with the blood of many other species.

It has not been definitely determined whether the L.E. cell factor is used up in a quantitative manner by its nucleolytic action. Some workers feel that its activity is actually enhanced by successive exposures to blood cells. It should be pointed out, however, that there are still many unknowns and variables surrounding the L.E. cell phenomenon and all methods used to quantitate the L.E. factor must be interpreted with caution. The most commonly used method of quantitation is determination of the proportion of L.E. cells among a definite number of observed, potentially phagocytic blood cells (neutrophils, eosinophils, and monocytes). This method, however, is crude at best



FIG. 8 (*below*)—Nucleophagocytosis, present in many preparations of bone marrow or blood The active phagocyte in this field is a small histiocyte which has engulfed a nucleus, probably of lymphocytic origin Note that the chromatin pattern of the engulfed nucleus is well preserved and that there is no difficulty in identifying it There is a tendency to increased density about the rim of this nucleus but no visible evidence of chromatolysis The normal appearing nucleus of the histiocyte is pushed to the periphery of the cell. This, I feel, is only a nonspecific defense reaction of the body phagocytes, engulfing nuclei which have become damaged, opsinized, or otherwise rendered susceptible to phagocytic activities The L E cell reaction, however, is very likely identical, except for the specific type of nuclear damage suffered by the engulfed nuclear mass L E cell factor has produced this specific chromatolysis (depolymerization of the desoxyribose nucleic acid) and the chromatolysis must be recognized to distinguish the L E. cell phenomenon from active nucleophagocytosis

and only very large variations are probably significant At the moment, it is most important for a clinician to know whether the result of an examination was positive or negative, but the information is not always reliable; specifically, the examiner must decide, on morphologic grounds, that the biochemical change of depolymerization of the desoxyribose nucleic acid of the affected nucleus has taken place. Because of uncertainties inherent in a "one cell diagnosis," it is desirable that the entire L.E. cell phenomenon be observed in a diagnostic preparation.

The quantitative potential of the L.E. cell factor (increased amount? increased activity of same amount?) is greatly increased by coagulation, or by some substance associated with coagulation, of the blood. In other words, many more L.E. cells are formed when blood or bone marrow is allowed to coagulate and stand than when an aliquot part of blood is kept from coagulating by the introduction of heparin or by the use of a silicone-coated apparatus in the procedure. In fact, one can almost state that if extreme care is used to prevent initiation of coagulation, L.E cells will not be formed, this suggests that some substance in the coagulation process may be the agent which transforms L E. factor into L E. cell factor. On purely theoretic grounds, I feel that this substance is associated with blood platelets and their degradation products

Recent investigations suggest that the L.E cell factor inactivates an inhibitor of desoxyribonuclease which normally occurs within polymorphonuclear leukocytes, thus permitting depolymerization of the desoxyribose nucleic acid in the nucleus of the victimized cell

In any consideration of the enigmatic L E cell factor, the work of Moolten and Clark (38) must be given a prominent place These workers isolated a virus from each of 6 patients with systemic lupus erythematosus, in each case, after multiple successive incubations in chick embryos, they were able to reproduce the L E cell phenomenon by mixing the virus culture with normal blood cells. In one instance, a volunteer was vaccinated with the dead or attenuated virus and produced antibodies which prevented formation of L E. cells.

NUCLEOLYSIS AND LYSED NUCLEAR MASSES

As has been stated previously, the fundamental step in the L E. cell phenomenon is lysis of the nuclei of body cells, particularly of



Fig 9 (*above*) —Another example of nucleophagocytosis by a small histiocyte In this instance, however, the engulfed nucleus is showing degenerative changes, there are vacuoles in the hyaloplasm and condensation of chromatin about the rim of the mass In the stained preparation, a reddish purple reaction, indicating a change in pH, was evident

Fig 10 (*below*) —Nucleophagocytosis by a bilobed, neutrophilic, polymorphonuclear leukocyte Note the intact nuclear pattern of this engulfed leukemic cell nucleus Preparation from bone marrow in a case of acute leukemia

and only very large variations are probably significant. At the moment, it is most important for a clinician to know whether the result of an examination was positive or negative, but the information is not always reliable, specifically, the examiner must decide, on morphologic grounds, that the biochemical change of depolymerization of the desoxyribose nucleic acid of the affected nucleus has taken place Because of uncertainties inherent in a "one cell diagnosis," it is desirable that the entire L.E. cell phenomenon be observed in a diagnostic preparation

The quantitative potential of the L E. cell factor (increased amount? increased activity of same amount?) is greatly increased by coagulation, or by some substance associated with coagulation, of the blood. In other words, many more L E cells are formed when blood or bone marrow is allowed to coagulate and stand than when an aliquot part of blood is kept from coagulating by the introduction of heparin or by the use of a silicone-coated apparatus in the procedure. In fact, one can almost state that if extreme care is used to prevent initiation of coagulation, L.E. cells will not be formed, this suggests that some substance in the coagulation process may be the agent which transforms L.E. factor into L E cell factor. On purely theoretic grounds, I feel that this substance is associated with blood platelets and their degradation products.

Recent investigations suggest that the L E cell factor inactivates an inhibitor of desoxyribonuclease which normally occurs within polymorphonuclear leukocytes, thus permitting depolymerization of the desoxyribose nucleic acid in the nucleus of the victimized cell.

In any consideration of the enigmatic L E cell factor, the work of Moolten and Clark (38) must be given a prominent place These workers isolated a virus from each of 6 patients with systemic lupus erythematosus, in each case, after multiple successive incubations in chick embryos, they were able to reproduce the L E cell phenomenon by mixing the virus culture with normal blood cells. In one instance, a volunteer was vaccinated with the dead or attenuated virus and produced antibodies which prevented formation of L E. cells

NUCLEOLYSIS AND LYSED NUCLEAR MASSES

As has been stated previously, the fundamental step in the L E cell phenomenon is lysis of the nuclei of body cells, particularly of

and other large elements in bone marrow preparations. This agglutination phenomenon is an aid to the investigator, for scanning of a preparation under low power objective often will give productive fields for study under the oil immersion objective, with the result that L.E. cells will be identified and the report will be positive.

The act of engulfing a lysed nuclear mass often can be observed on fixed preparations, even 2 cells dividing the same mass as it is taken into their respective cytoplasmic vacuoles. Under supravital staining this part of the phenomenon can be watched, as can the progressive nucleolytic change.

The phagocytic cells are most commonly neutrophilic polymorphonuclear leukocytes, although eosinophils are not uncommon. In the clotted preparations, phagocytic monocytes and small histiocytes are now commonly seen, and it is believed that this is probably because of the longer period available to the less motile phagocytes. In a 2-hour clot preparation, for example, the more slowly moving monocytes and histiocytes can wander through the fibrin network and pick up multiple lysed nuclear masses. In some preparations which are strongly positive for the L.E. cell phenomenon, single phagocytes can be seen which contain multiple inclusion bodies as well as numerous lysed nuclear masses held at their periphery (a finding reminiscent of erythrophagocytosis in hemolytic states).

Because the word "donor" so widely connotes blood transfusion, it should be emphasized that when used in the next paragraphs transfusion is not implied, once again, it is an *in vitro* phenomenon with which this discussion is concerned. Blood and bone marrow cells of an individual with systemic lupus erythematosus, or cellular elements from human or animal donors not afflicted with the disease, will produce the L.E. cell phenomenon when exposed to the lytic action of the L.E. cell factor contained in either plasma or serum of the patient. There have been reports that not all donor cells act alike in the above circumstances, this suggests individual susceptibility to lysis and varying phagocytic ability. There is also some species difference, for example, actual phagocytosis to produce an L.E. cell is uncommon with dog marrow preparations while the rosette formation is outstanding and diagnostic. Furthermore, in an occasional preparation numerous lysed nuclear masses are found and yet there is no phagocytic activity resulting in formation of L.E. cells. There is still much work to be done on this aspect of the problem.

cells of blood and bone marrow. This process can be identified when the chromatin of the nucleus loses its structural pattern, becomes "smoothed out" and takes on a homogeneous appearance. In addition, the affected part of the nuclear mass loses its ability to take stain properly; it appears pale and rather dirty in contrast to normal nuclear staining in the same field. As this lytic process progresses, the lysed nuclear mass swells and, if the mass is intracellular, it tends to push the normal nuclear remains to the periphery of the cell Eventually these afflicted cells rupture and a free, lysed nuclear mass remains. These lysed nuclear masses are very prominent in preparations if 2 hours of clotting has preceded the processing of the material; swollen bilobed, round single-lobed, and ocassionally, entire lysed L.E. cells can be seen. The impression is that most of the lysed nuclear masses encountered in laboratory preparations in which blood is used for demonstrating the L.E cell phenomenon come from polymorphonuclear leukocytes. It is often possible to see part of a nucleus which has undergone lysis and a sharp line of demarcation between the lysed and normal chromatin pattern

An occasional L E cell can be seen in which the engulfed, lysed nuclear body evidently contained an excess, or free quantity, of L.E cell factor which in turn has started lysis of the host nucleus. Rather than look only for L E cells in preparations, one should study this lytic process in all of its manifestations to be sure that it precedes and accompanies phagocytosis Nucleophagocytosis without lysis can be, and is, a source of confusion and false positive reports!

PHAGOCYTOSIS AND FORMATION OF ROSETTES

After the nucleus of a cell has begun to undergo lytic changes it becomes a foreign body and, under proper conditions, is engulfed by a phagocytic cell The lysed nuclear mass exerts a chemotactic effect on wandering phagocytes which move into the field and agglutinate about the material in an attempt to engulf it. On fixed preparations, these agglutinated clumps of cells are easily found. Their characteristics are a central core of lysed nuclear material and a surrounding group of phagocytes making up the rosette of Haserick (19).

Since such clumps of cells tend to cling together as a mass larger than individual cells, they usually are carried to the fringe or feather-edge of the smear, as are metastatic cells, megakaryocytes, osteoclasts,

NUCLEOPHAGOCYTOSIS (TART CELL FORMATION)

At this point it should be pointed out that there are probably numerous situations in which the defense mechanisms of the body are stimulated and various antibodies, agglutinins, opsonins, and lysins are increased, together with an associated increase in phagocytosis. Phagocytosis of leukocytes by large phagocytic reticuloendothelial cells is a common finding in preparations of bone marrow, spleen, and liver of some individuals ill with a variety of conditions. Phagocytosis of leukocytes or of their damaged (opsonized, traumatized, dead) nuclei is accomplished also by neutrophils, monocytes, and small histiocytes. *Such cells with phagocytized, damaged nuclei as inclusion bodies, can be mistaken for L E cells* The phagocytic cells are most commonly small histiocytes, and preparations of bone marrow are the ones which most frequently yield examples of this nucleophagocytosis. However, examples are not uncommonly found in laboratory preparations made to determine the presence or absence of the L E. cell phenomenon

The phagocytized nucleus seen in nucleophagocytosis (tart cells) can usually, but not always, be distinguished from the lysed nuclear mass of an L E. cell. The following observations have been made by others as well as by me (*1*) The phagocytized nucleus usually maintains an intact chromatin pattern and, unlike the inclusion body in an L.E. cell, is not the swollen, lysed mass with decreased staining quality (*2*) The phagocytized nucleus tends to become vacuolated and the chromatin to become more dense. (*3*) The circumference of the phagocytized nucleus is commonly "condensed," giving the impression of a thick rim (*4*) Eosinophilic staining of the nucleus may be more brilliant after ingestion than before, probably because of changes in pH incident to digestive activity within the vacuole of the phagocyte.

Nucleophagocytosis, as well as phagocytosis of various tissue cells, has been produced by injecting the antigen into test animals and stimulating antibody formation Serums of such animals will produce nucleophagocytosis when mixed with the donor's cells Whether such a procedure will produce a nucleolytic agent capable of initiating or consummating the depolymerization of the desoxyribose nucleic acid is as yet unproved, so far as I know Consequently, I feel that L E. cells have not been produced except by reaction with the L E cell factor, which is specific for systemic lupus erythematosus I have always stressed the fact that in some cells the most experienced observer cannot distinguish the lysed nuclei of systemic lupus erythematosus



Fig 11 (*above*) —Nucleophagocytosis by 2 neutrophilic, polymorphonuclear leukocytes Note the characteristic preservation of chromatin pattern, condensation of chromatin around the periphery, and the tendency of vacuoles, or clear areas, to develop in the hyaloplasm of the engulfed nuclei

Fig. 12 (*below*) —Clump of neutrophilic polymorphonuclear leukocytes agglutinated about scattered, lysed, chromatin material In the clump is a typical L E. cell which contains a smooth homogeneous, lysed, chromatin mass On the opposite side of the clump is a cell, the engulfed nuclear mass of which still has a nuclear pattern and chromatolysis is not complete If the latter cell were seen alone, it would present a problem of identifying it either as an L E cell or simply as a cell displaying nucleophagocytosis

from the damaged, opsoninized, partially digested, engulfed nuclear mass which is due to entirely different causes. It is in the study of a population of cells that the difference becomes apparent.

L.E. CELL

[illegible]

indicator of the end point of this biochemical reaction which seems to be characteristic of systemic lupus erythematosus under the conditions stated I am confident that some day the L.E. cell will be displaced by a more accurate and dependable indicator; but until then, the accuracy of diagnosis will continue to depend on the experience and interpretive judgment of the trained microscopist

The L E cell may be defined, then, as a phagocytic cell within the cytoplasm of which there is an inclusion body consisting of nuclear material that has undergone lysis (depolymerization of the desoxyribose nucleic acid). The phagocytic cell most frequently observed is the neutrophilic polymorphonuclear leukocyte, although eosinophilic granulocytes are not uncommon. The more slowly moving phagocytes (histiocytes and monocytes) become increasingly prominent as preparations are made by procedures which take increasingly more time. The inclusion body is usually a relatively smooth, homogeneous nuclear mass, staining less prominently than the host nucleus (although it may be in any stage of chemical change, with associated morphologic change); it is most frequently derived from neutrophils and lymphocytes which have succumbed to the effects of the L.E. cell factor. On occasion, there may be more than one inclusion body.

ARTIFICIAL PRODUCTION OF L E. CELLS

Since the L.E cell is the result of a biochemical reaction, it would seem reasonable to suppose that other chemical or biologic agents than the L E. cell factor could reproduce the L E. cell phenomenon. Under experimental conditions this has been accomplished by Inderbitzin (24), who utilized the anticoagulants sodium polymetholsulfonic acid (Liquoid Roche) and the sodium salt of polyvinyl alcohol polysulfonic acid ester (PVAS Roche) as the nucleolytic agents.

In the first instance (Liquoid Roche), the inclusion body usually



FIG 13 (*above*) —A phagocytic, neutrophilic, polymorphonuclear leukocyte with 2 inclusion bodies One inclusion body has all of the characteristics of an L E cell inclusion, while the material in the other cytoplasmic vacuole still has a good chromatin pattern The latter mass shows swelling of the chromatin particles, reduced staining reaction, lack of peripheral chromatin condensation, and other characteristics which to the experienced examiner suggest activity typical of the L.E cell factor

FIG. 14 (*below*) —Field showing 2 L E cells with lysed chromatin masses within their cytoplasm, the lytic process is not yet complete In the third L E cell are 3 separate inclusion bodies showing all degrees of chromatolysis



FIG 17 (*above*) —High power field from a 2-hour blood-clot preparation positive for the L E cell phenomenon There are 4 L E cells, 3 of which are monocytes and the fourth a neutrophilic granulocyte Note the bilobed, lysed, nuclear mass that is free in the field Note the monocyte in one corner of the field containing a nuclear mass, while around its periphery a number of lysed nuclear masses are being held (similar to spherocytes being held at the periphery of phagocytic cells as observed in erythrophagocytosis in an occasional case of acute infectious hemolytic anemia) The other phagocytic cells have picked up masses of lysed nuclear material of varying size

FIG 18 (*below*) —*A*, in the 2-hour clot preparations, sometimes long, swollen, strands of nuclear material (thick spiremes) can be seen engulfed *B*, 2 phagocytic neutrophils which have picked up and ingested opposite ends of an elongated, lysed nuclear mass



FIG 15 (*above*) —Low power view of the featheredge of a 2-hour blood-clot preparation positive for the L E cell phenomenon Note agglutination of phagocytic cells around the lysed nuclear masses, rosette formation, L E cells, as well as free, lysed nuclear masses which have not yet been attacked by phagocytes

FIG 16 (*below*) —High power view illustrating the progressive chromatolytic changes of systemic lupus erythematosus as observed in a preparation from a 2-hour blood-clot procedure The nucleus of a neutrophilic polymorphonuclear leukocyte is just beginning to swell The cell itself is still intact To the side of this cell is a swollen, partially lysed, bilobed nuclear mass surrounded by scattered neutrophilic granules from the ruptured cell Opposite these 2 cells are 2 lysed nuclear masses so placed as to arouse suspicion that this is a lysed and ruptured L E cell. The rounded mass was probably the original inclusion body of the L E cell, while the darker, possibly bilobed nuclear mass could have been the L E cell nucleus, now they are both free, lysed nuclear masses In the opposite corner is an L E cell with a double inclusion body

drug may or may not be a factor in the formation of "false" L E. cells. Occasionally, an individual with clinical rheumatoid arthritis and treated with cortisone has yielded clotted blood preparations in which L.E. cells have been found in small numbers Only time and more knowledge of the fundamental changes involved in clinical rheumatoid



FIG 19 –Erythrophagocytosis in a 2-hour blood-clot preparation which was negative for the L E cell phenomenon If, as in one of these cells, the engulfed erythrocytes had been polychromatophilic in their staining quality, this field might have been mistaken for a group of L.E cells

arthritis and systemic lupus erythematosus will tell whether or not these are "false positive reactions"

The problem of the relationship, if any, between drug reactions and the L E cell phenomenon requires further investigation.

EXPERIMENTAL PRODUCTION OF L.E. CELLS IN ANIMALS

Castillo, Fernández, and Remedios reported the production of L E cells in the blood of guinea pigs which had received repeated injections of 2 to 5 cc. of whole blood obtained from patients with systemic lupus erythematosus Examination of tissue from these experimental animals revealed changes similar to those found in material obtained from human victims of systemic lupus erythematosus Interestingly, these changes could be induced only in female guinea pigs.

was found to be composed of disintegrated thrombocytes, and when it was of nuclear origin the process could be recognized as nucleophagocytosis and not nucleolysis. To quote: " . . . . We have long been able to produce phagocytic phenomena by the white cells in which the phagocytic cells morphologically resembled the L.E. cells. However, destruction of the cells and decomposition of the nuclei into amorphic acidophilic material, with subsequent phagocytosis, was lacking. In our experiments, we were still able to identify the origin of the material which had been engulfed. The qualitative changes always were shown by using the May-Grunwald-Giemsa stain." It was also found that this reaction was independent of any serum factor.

Experiments with PVAS, however, yielded a cell indistinguishable from the L.E. cell of systemic lupus erythematosus. Furthermore, a serum factor took part in the reaction, and more specifically this factor was found to reside in the gamma globulin fraction (as the L.E cell factor does). However, this "artificial" L.E cell factor could be produced only by heating the gamma globulin fraction (or whole serum) with PVAS for about 30 minutes at 59 C. It is also of interest that if coagulation of blood was inhibited by heparin before PVAS was added, the L.E cell phenomenon did not occur.

It seems superfluous to point out that what has just been described is purely an experimental procedure and in no way lessens the value of the true L.E cell phenomenon as a diagnostic laboratory procedure. However, doubts have been expressed by interested physicians As Inderbitzin has said: "Our experiments with cellular changes morphologically identical with genuine L.E. cells can only be produced in a serum that has been subject to such physical processes that it is impossible for it to occur in the organism. In addition PVAS is a foreign substance to the body." On the other hand, such an experimental approach may well lead to the unfolding of the true pathologic and etiologic factors involved in a mysterious disease

DRUG REACTIONS

It has been reported that L.E cells have been found in laboratory preparations of the blood of individuals with drug reactions, particularly reactions to penicillin. I have not had this experience, although I have noted nucleophagocytosis in the circumstances mentioned. I have not investigated this matter sufficiently to permit further comment here

However, I have had experience with another situation in which a

4 The serum is decanted and discarded unless the observer wishes to keep it for other purposes.

5. The remaining clot is forced through a screen of fine mesh to defibrinate it and the resulting fluid portion, containing the cellular elements, is collected in a chemically clean, sterile Petri dish.

6 The defibrinated blood is transferred to Wintrobe hematocrit tubes for centrifugation, 2 tubes are best in case the resulting buffy cellular layer is sparse

7. Centrifugation is carried out at 1,500 to 1,800 rpm for about 5 minutes.

8 The serum is removed, the buffy cellular layer is collected with a pipet and is transferred to a small paraffined receptacle (paper boat).

9 From the remixed buffy-coat layer, drops of suitable size are transferred to chemically clean glass slides or coverslips and smears are made in the usual fashion.

10. Staining is by the usual blood smear technic and examination is made for the L E. cell phenomenon



Lee's Blood Clot Method

1 Obtain about 2 cc. of venous blood by venipuncture from the suspected subject

2. Allow blood to coagulate in a clean tube and stand for 2 hours

3 Puncture and traumatize the clot with wooden applicator sticks, so that some blood escapes into the serum.

4 Remove the clot

5 Centrifuge the serum.

6 Make smears from the sediment and stain in the usual way for microscopic study

Other Methods

Marten and Blackburn (36) describe a simple method in which defibrination and coagulation occur while the blood is agitated in a 1 oz (about 30 cc) bottle In the bottle is a bent paper clip about which the fibrin collects Centrifugation of the material then yields buffy-coat layers for the rest of the procedure

Weisberger, Meacham, and Heinle (52) have devised a method in which bovine fibrinogen is utilized to hasten sedimentation of red cells in heparinized blood. Then the supernatant fluid is centrifuged to obtain cellular components for study.

Confirmation of these observations by other workers, and extension of the experimental studies, will be watched with great interest. Duplication of systemic lupus erythematosus in experimental animals, and of the diagnostic phenomenon of the L.E. cell in relation to these animals, would be a great boon to medical research

DIAGNOSTIC PROCEDURES BASED ON L.E. CELL PHENOMENON

Once the fundamentals of the L.E. cell phenomenon are understood, it can be utilized in many ways as a diagnostic laboratory procedure Three ingredients are necessary for a productive procedure: (1) a source of the L.E. cell factor, (2) nucleoprotein with which the L.E. cell factor reacts, and (3) phagocytic cells to engulf the lysed nuclear material resulting from interaction of the L.E. cell factor and the nucleoprotein.

Since the reaction is a biochemical one, sufficient time must elapse, and proper temperature must be maintained, to permit optimal reaction Most of these procedures are carried out at room temperature or in a water bath at 37 C. At this temperature, the minimal time for optimal results is probably around 30 minutes, 1 to 2 hours can elapse without serious degenerative changes occurring

The L.E. cell phenomenon not only will occur in clotted blood but is enhanced by such clotting The latter fact has been utilized in the procedure routinely employed in our laboratory; the procedure has been modified from Zimmer's original investigative work by the introduction of a defibrinating screen devised by Magath Another clotted blood technic, devised by Lee (30) in the course of his investigations, is simple and reliable These procedures, outlined below, have the advantage that all elements are supplied by the individual suspected of having systemic lupus erythematosus.

Zimmer's 2-Hour Blood Clot Technic (Modified)

1. Approximately 10 cc of blood is obtained by venipuncture from the individual suspected of having the disease

2. This blood is placed in a sterile, dry, chemically clean centrifuge tube of sufficient size

3 The blood coagulates and stands in the supported tube for about 2 hours at room temperature

In my hands, preparations in which serum and plasma are utilized have seemed to lack the degree of reactivity found in coagulated material. However, procedures in which plasma or serum is utilized and nucleoprotein and phagocytic cells are obtained from a donor have two advantages. (1) Suspected plasma (serum) can be sent for examination to distant points when additional opinions are desired, or it can be kept frozen for long periods if further use is contemplated. (2) Plasma (or serum) permits use of various control measures, such as comparison of the suspected material with normal and with known L.E. plasma, or the making of separate determinations with cells of each of several donors. The latter may prove to be important, as some donor cells have been reported to be resistant or nonreactive to the L.E. cell factor.

Of the methods in which plasma is utilized, Haserick's (19) probably is best known. He had access to fresh dog marrow as a source of the reacting nucleoprotein and of the phagocytic cells. With this combination, however, L.E. cells are not commonly found, while rosette formation is outstanding and is accepted as evidence of a positive L.E. cell preparation.

Barnes, Moffett, and Weiss (1) have employed two procedures in most of their work. In the first, heparin is used to prevent coagulation, and the plasma of the suspect is mixed with buffy-coat cells from the heparinized blood of healthy donors. In the second procedure, the serum of the suspect is obtained by coagulation of venous blood, while buffy-coat cells are obtained by defibrination of the donor's blood with a glass rod.

The simplest of plasma procedures, of course, is that utilizing the whole blood of the suspect. Heparin is used as an anticoagulant for a venous blood sample, and the sample is centrifuged to utilize the buffy coat of leukocytes for blood smear preparations. The major disadvantage of this procedure, besides loss of the coagulation effect, is the leukopenia so often present in the blood of people suffering from lupus erythematosus. Leukopenia also may be a disadvantage in a clotted blood technic. When this has occurred, we have used the serum from the 2-hour blood-clot procedure, mixed with cells obtained from a donor.

My concept of the L.E. cell phenomenon, as distinguished from nucleophagocytosis, is derived from the morphologic changes demonstrated in Figures 1–19. The technic of the 2-hour blood-clot procedure is shown in Figure 20.

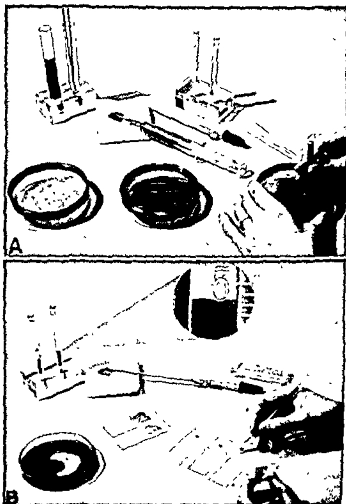


Fig 20 – Technic of the 2-hour blood-clot procedure. *A*, 10 cc of venous blood from an individual suspected of having systemic lupus erythematosus is allowed to coagulate in a sterile, chemically clean, tube and to stand for about 2 hours at

ferred to chemically clean glass slides, then smeared in the usual fashion of preparing blood smears These preparations are then stained in the usual way (Wright's stain) and studied for evidence of the L E cell phenomenon

enon induced by lupus erythematosus plasma, J Invest Dermat 13 47, 1949

21. Haserick, J R, and Lewis, L A. Blood factor in acute disseminated lupus erythematosus II Induction of specific antibodies against L E factor, Blood 5 718, 1950
22. Haserick, J R, and Sundberg, R D. The bone marrow as a diagnostic aid in acute disseminated lupus erythematosus Report on the Hargraves' "L E" cell, J Invest Dermat 11 209, 1948
23. Haserick, J R, Lewis, L A, and Bortz, D W. Blood factor in acute disseminated lupus erythematosus I Determination of gamma globulin as specific plasma fraction, Am J M Sc. 219 660, 1950.
24. Inderbitzin, T. The experimental production of leukocytic changes in normal blood similar to LE-cells and morphologically identical with LE-cells, J Invest Dermat 20 67, 1953
25. Jamba, M Hematological aspects of lupus erythematosus disseminatus, J A M A 152 1358, 1953
26. Klemperer, P Pathology of Systemic Lupus Erythematosus, in McManus, J. F A (ed) *Progress in Fundamental Medicine* (Philadelphia Lea & Febiger, 1952)
27. Kurnick, N B, *et al* Studies on desoxyribonuclease and systemic lupus erythematosus Non-participation of serum desoxyribonuclease in the "L E. phenomenon," J Clin Invest 31 1036, 1952
28. Kurnick, N B, *et al* A specific inhibitor for human desoxyribonuclease and an inhibitor of the lupus erythematosus cell phenomenon from leucocytes, J Clin Invest 32 193, 1953
29. Kurnick, N B, *et al* The role of desoxyribonuclease and a nuclease inhibitor from leucocytes in the lupus erythematosus cell phenomenon, J Clin. Invest 31 645, 1952
30. Lee, S L A simple test for L E cells, Am J Clin Path 21 492, 1951
31. Lee, S L, Michael, S R, and Vural, I L The L E cell, Bull New York Acad Med 26 266, 1950
32. Lee, S L, Michael, S R, and Vural, I L The L E (lupus erythematosus) cell, Am J Med 10 446, 1951
33. Magath, T B, and Winkle, V Technic for demonstrating "L E" (lupus erythematosus) cells in blood, Am J Clin Path 22 586, 1952
34. Marmont, A Le cosidette angiomesenchimopatic reattive diffuse [The so-called angiomesenchymopathic diffuse reaction], 52d Congresso della Società Italiana di Medicina Interna, 1951
35. Marmont, A Valore e significato biologico del cosidetto "fenomeno L E" nell'eritematode sistemico acuto [The value and biologic significance of the so-called "L E phenomenon" in acute systemic erythematosus], Minerva med 44 (pt 1) 80, 1953
36. Marten, R H, and Blackburn, E K A simple method for the detection of L E cells, J Clin Path 6 89, 1953
37. Mathis, H B A simple office procedure for demonstrating lupus erythematosus cells in peripheral blood, Blood 6 470, 1951
38. Moolten, S, and Clark, E Virus studies in systemic lupus erythematosus Unpublished data.

REFERENCES

1. Barnes, S. S., *et al.*: Studies on L.E. phenomenon, Arch. Dermat. & Syph. 62:771, 1950.
2. Barnes, S. S., Moffett, T. W., and Weiss, R. S.: Demonstration of the L.E. cell in the absence of anticoagulant, J. Invest. Dermat. 14:397, 1950.
3. Berman, L., *et al.*: So-called "lupus erythematosus inclusion phenomenon" of bone marrow and blood. Morphologic and serologic studies, Am. J. Clin. Path. 20:403, 1950.
4. Bertrand, L.: Les cellules de la lupo-erythémato-viscérite maligne (L.E. phénomène). Intérêt de leur recherche sur la couche leucocytaire du sang concentré, Presse méd. 60:929, 1952.
5. Butterworth, C. E., Jr.: The production of L.E. cells in normal individuals, J. Clin. Invest. 32:558, 1953.
6. Cajano, A.: Personal communication.
7. Carrerea, A., Reid, V., and Kurnick, N. B.: Differences in susceptibility of polymorphonuclear leucocytes from several species to alteration by systemic lupus erythematosus serum, J. Clin. Invest. 32:559, 1953.
8. Castillo, P., Fernández, L., and Remedios, V. G.: Celulas "L.E." en el curiel. Notra preliminar (The production of the L.E. cell in guinea pigs), Arch. med. int. 12:173, 1952.
9. Chomet, B.: Personal communication.
10. Finch, S. C., Ross, J. F., and Ebaugh, F. G., Jr.: Immunologic mechanisms of leukocyte abnormalities, J. Clin. Invest. 32:567, 1953.
11. Gold, S. C.: Some observations on the pathogenesis of systemic lupus erythematosus, J. Invest. Dermat. 19:333, 1952.
12. Gonyea, L. M., Kallsen, R. A., and Marlow, A. A.: The occurrence of the "L.E." cell in clotted blood, J. Invest. Dermat. 15:11, 1950.
13. Gross, L.: The cardiac lesions in Libman-Sacks disease with a consideration of its relationship to acute diffuse lupus erythematosus, Am. J. Path. 16:375, 1940.
14. Gueft, B.: Depolymerization of nucleic acid in acute disseminated lupus erythematosus, Arch. Dermat. & Syph. 61:892, 1950.
15. Hargraves, M. M.: Production in vitro of the L.E. cell phenomenon. Use of normal bone marrow elements and blood plasma from patients with acute disseminated lupus erythematosus, Proc. Staff Meet., Mayo Clin., 24:234, 1949; The L.E. cell phenomenon, ibid. 27:419, 1952.
16. Hargraves, M. M., Richmond, H., and Morton, R.: Presentation of two bone marrow elements: The "tart" cell and the "L.E." cell, Proc. Staff Meet., Mayo Clin., 23:25, 1948.
17. Haserick, J. R.: Blood factor in acute disseminated lupus erythematosus, Arch. Dermat. & Syph. 61:889, 1950.
18. Haserick, J. R.: Simulation of the lupus erythematosus phenomenon by materials of fungal origin, J. Invest. Dermat. 16:211, 1951.
19. [illegible] acute disseminated [illegible]
20. [illegible] inclusion phenom-

Biopsy Studies of the Liver and Kidney

POUL IVERSEN, M. BJØRNEBOE, AND N. B. KRARUP

From Medical Department III, Kommunehospitalet, Copenhagen, Denmark

LIVER BIOPSY

THE LIVER IS an organ that seems well suited for biopsy. It is a large organ of uniform structure, localized rather superficially in the abdomen. The surface of the lobule, the anatomic functional unit of the liver, measures only a few square millimeters. Since many of the hepatic pathologic processes are diffuse in nature, a minute tissue sample will generally suffice to give a representative picture of the condition of the entire organ (111)

Diagnostic and therapeutic punctures of abscesses and cysts in the liver have been practiced in clinical medicine for many years The attempt by clinicians to remove with syringe and trocar a small specimen of liver tissue for histologic examination followed naturally. The first attempts were made in the last century (57) Schupfer (87), in 1907, is generally considered to be the first to have aspirated tissue fragments which permitted a more thorough pathoanatomic diagnosis. These investigations were continued by Bingel (6), who performed 100 punctures, with 2 deaths, by Olivet (68), with 2 deaths in 140 cases, and, in smaller series, by Scalabrino (84) and by Waldenstrom (110). In 1939, Baron (4) reported the results of 48 biopsies in 35 patients (1 death). He introduced the needle transperitoneally, below the costal margin He found the method especially valuable in the diagnosis of cancer, for detecting cirrhosis it was useless, probably because with this method the tough tissue could not be removed by aspiration. The same year, Iversen and Roholm (42) described their

39. Pisciotta, A. V., *et al*: Acute hemolytic anemia in disseminated lupus erythematosus. Treatment with cortisone, Report of case, Am. J. Clin. Path. 21, 1139, 1951.
40. Rebuck, J W., and Berman, L: Experimental production of the L.E phenomenon in the skin of man, Proc. Soc. Exper. Biol & Med. 75:259, 1950.
41. Rohn, R. J., and Bond, W. H.: Some supravital observations on the "L.E" phenomenon, Am J. Med 4.422, 1952
42 Seaman, A. J., and Christerson, J. W.: Demonstration of L E cells in pericardial fluid, J A M A. 149.145, 1952
43. Smith, P. A. J: The "L.E. cell" and its significance, Brit. J. Dermat 64 10, 1952.
44 Stevens, J E, *et al.* The "L E cell": A phenomenon of diagnostic value in acute disseminated lupus erythematosus, Virginia M. Month 78 590, 1951.
45 Stich, M. H; Feldman, F., and Morrison, M Pre-L.E cell A stage in development of the L E. phenomenon, Arch Dermat & Syph 65 581, 1952.
46 Suksta, A, and Conley, C. L · Some observations on L.E. cell, J. Lab & Clin Med. 37 597, 1951.
47. Sundberg, R. D., and Lick, N. B.: "L E." cells in the blood in acute disseminated lupus erythematosus, J. Invest Dermat. 12 83, 1949.
48. Van Doormaal, T. A J, and Schreuder, J. T. R · Über die sogenannte Erythematodeszelle und deren Vorkommen in der Pleuraflussigkeit bei einer an "Lupus erythematodes disseminatus acutus (subacutus)" leidenden Patientin, Dermatologica 101 167, 1950.
49 Walsh, J R, and Egan, R L The reliability of the L E test, New England J Med 246 775, 1952
50 Walsh, J R, and Zimmerman, H J.: The demonstration of the "L.E" phenomenon in patients with penicillin hypersensitivity, Blood 8.65, 1953
51. Watson, J B., O'Leary, P A, and Hargraves, M M.: Neutrophils resembling L.E. cells in artificial blisters, Arch. Dermat. & Syph 63·328, 1951
52 Weisberger, A S, Meacham, G C, and Heinle, R. W · Simple method for demonstrating the "L.E" phenomenon in peripheral blood, J Lab & Clin Med 39 480, 1952
53 Zimmer, F E, and Hargraves, M M. The effect of blood coagulation on L E cell formation, Proc Staff Meet, Mayo Clin 27 424, 1952
54 Zimmerman, H J, Walsh, J R, and Heller, P Production of nucleophagocytosis by rabbit antileukocytic serum, Blood 8.651, 1953

seconds, and a cylinder of liver tissue, 2 × 15 mm., is generally obtained.

In most cases, the tissue is sucked into the syringe as the cannula is withdrawn. The specimen is then dealt with like other histologic preparations, or it can be subjected to biochemical study. The small size of the specimen allows rapid fixation, the advantages of which are obvious. The procedure, in use now for some 15 years, has proved highly satisfactory.

With the gradually increasing interest in this diagnostic method, a number of modifications of the original procedure have been introduced (16, 23, 28, 31, 34, 36, 60, 74, 89, 101). However, in our opinion, the various changes in the instrument are of comparatively minor importance so long as the investigator is sufficiently familiar with his own instrument.

As Diagnostic Procedure

Despite the large number of liver function tests, liver disease often poses extremely difficult diagnostic problems. In long-standing cases of jaundice, particularly, it is often impossible to establish the diagnosis with certainty. This is all the more unfortunate since the correct treatment depends on an exact diagnosis. The differential diagnosis in a case of long-standing jaundice must usually establish whether the jaundice is parenchymatous or obstructive in type, and, in the latter case, whether the obstruction is caused by a calculus or a tumor. Differentiation of other liver diseases, such as cirrhosis or cancer, may also be fraught with difficulty.

Aspiration biopsy has clearly defined the histopathology of hepatitis and of obstructive jaundice. While the histologic lesions in the two conditions differ markedly, the differences are most pronounced early (first few weeks of the disease), and tend to become less clear with time. Degenerative changes in the parenchymal cells and an increase in connective tissue develop in long-standing biliary stasis, this may render the differentiation between parenchymatous liver disorders and biliary obstruction difficult. It is in this field that the histologist is aided by his wealth of experience. The clinician, too, must bring considerable knowledge to his discussion of the case with the pathologist, for otherwise the value of the liver biopsy is considerably diminished. The differential diagnosis is hardly ever simple, and in some cases the difficulties may be such that the diagnosis remains in doubt.

technic, which consisted of a combination of boring, cutting, and aspiration so as to secure a large enough sample of tissue to permit a thorough investigation. The report (75) on their investigations of the histopathology in acute hepatitis aroused considerable interest. The following years saw the appearance of a large number of reports on liver biopsy (2, 19, 27, 33, 36, 38, 55, 60, 65, 69, 86, 98, 100, 102, 103, 108, 114).

It is the purpose of the present discussion to survey briefly the scientific and diagnostic results that have been achieved by this method.

TECHNIC

The procedure we use routinely is the one originally described by Iversen and Roholm. The biopsy is always performed in the hospital The patient should be in bed, in a recumbent position Local anesthesia is used, and the few minutes until it becomes effective can be employed to teach the patient how to fix his muscles in a position of maximum expiration

The trocar, consisting of a cannula* 18 cm. long and 2 mm in diameter, with a tapering but not too pointed stilet, should be dry-sterilized The edge of the cannula is sharply ground and has 4 notches, so that it forms a wavy line. To minimize the risk of hemorrhage, it is important that the bore of the cannula should not exceed 2 mm.

The puncture is made in the posterior or in the midaxillary line immediately below the lower edge of the serratus anterior muscle The trocar thus passes through the pleura, diaphragm, and peritoneum before it reaches the surface of the liver

The skin is painted with iodine, a small incision is made with a pointed scalpel, and the trocar is introduced until it reaches the upper surface of the diaphragm (this is easily felt) The trocar is then withdrawn a little, the patient is asked to breathe deeply twice and thereafter to fix his muscles in a position of maximal expiration During this brief apnea the trocar is passed through the diaphragm, the stilet is withdrawn, and a 10 ml Record syringe with a locking mechanism on the piston is mounted The piston is pulled back and the cannula is passed 2 cm into the liver, at the same time, the cannula with syringe attached is turned several times around its axis, after which it is withdrawn rapidly The whole procedure takes only a few

* The Vim-Silverman cannula seems to be the one most widely used.

ful physical examination, and a judicious combination of liver function tests, can appreciably reduce the element of uncertainty.

RISKS OF THE METHOD

The physician has to decide whether the nature and frequency of complications in a given case of liver disease warrant the use of liver biopsy. Since the regenerative tendency of the liver is marked, the injury caused by biopsy is without major importance.

In a study of 297 biopsies, the site of puncture was examined at autopsy in 23 cases in which death occurred (for other reasons) between 3 and 31 or more days after the biopsy, in none of the 23 were any traces of the biopsy demonstrable (78). In 5 other cases, examined 10 hours, and 1, 5, and 9 days, respectively, after the biopsy, the stab wound could be seen, in 3 of them there was evidence of previous hemorrhage. Various complications were noted following liver biopsy. Some patients complained of a transient, rather pronounced pain, starting immediately after the biopsy; it was localized in the gallbladder area and radiated toward the shoulder. This pain presumably is caused by a lesion of one of the larger bile ducts,* and is particularly apt to occur if the biopsy is performed too far anteriorly. In other patients, a stinging pain on respiration, undoubtedly of pleural origin, developed in the right half of the thorax. A somewhat more intense pain in the right hypochondrium, which occurred in a few patients, may possibly have been due to a subcapsular accumulation of blood or bile

If the puncture is performed in the posterior or midaxillary line in the ninth intercostal space, the risk of injuring adjacent organs is practically excluded In our opinion, there is considerably more danger of such injury when the puncture is made below the costal margin. In 1 case of subcostal puncture, even a small bit of the mucous membrane of the colon was removed, fortunately, this produced no alarming symptoms (4).

There is no denying the possibility that tumor cells might be implanted in the stab wound (94). In most cases in which it might occur, however, this complication would not change the course of the disease. The dangers of air embolism are chiefly of a theoretic nature Although

*EDITORS' NOTE —Pain radiating to the shoulder could also arise from irritation of the diaphragm, which is perforated during the puncture, or from a subcapsular hematoma

Aspiration biopsy often yields a positive answer. Nevertheless, in certain conditions its value in differential diagnosis is limited, for instance, the hepatic changes in obstruction are the same, whether a calculus or a tumor is the cause. This is a minor drawback, however, since usually only a decision whether to operate or not is called for.

Biopsy is a valuable aid in the diagnosis of primary or metastatic cancer of the liver (86). Safdi *et al.* (82), who performed 357 biopsies in 308 patients without any untoward incident, found 9 cases of neoplasm which had not been diagnosed previously. In 32 other cases the biopsy diagnosis confirmed the clinical one, while in 12 the results of biopsy were negative, although the patients had a primary or secondary hepatic neoplasm A negative liver biopsy therefore does not exclude the possibility of cancer, as Svaar-Seljesaeter (97) emphasized In his opinion, aspiration biopsy is of greater diagnostic value in diffuse parenchymatous changes than in hepatic cancer.

Many investigators have stressed the importance of aspiration biopsy as an aid in differential diagnosis (65, 86, 89, 102, 115), although in some cases the results of biopsy have been misleading (29). In rare disorders, such as Boeck's sarcoid, which are particularly difficult to diagnose, biopsy is of special value (105), it has also been found helpful in the diagnosis of granulomatosis (86) and of miliary tuberculosis (20, 81)

Bjørneboe *et al* (7) have investigated the value of aspiration biopsy in cases requiring decision for surgical intervention Of 1,161 patients with hepatic disease admitted to hospital, the decision to operate on 46 was based on biopsy results, all other available diagnostic methods having failed to give a clear-cut answer In 4 of them the decision was proved to be wrong on operation: 1 case of cholelithiasis had been misdiagnosed as hepatitis, and 3 cases of hepatitis had been incorrectly diagnosed as obstructive jaundice Nevertheless, biopsy had reduced the diagnostic uncertainty from 4 per cent of the cases to about 0 5 per cent Despite the best possible combination of available liver function tests, even the experienced clinician finds that in 4 to 5 per cent of patients with jaundice the differential diagnosis cannot be established with certainty (96) Judging by the results reported by Bjørneboe *et al* (7), proper use of aspiration biopsy can considerably reduce this uncertainty

In summary, it can be said that while aspiration biopsy has not completely eliminated the uncertainty of differential diagnosis in protracted jaundice, its use, when combined with a detailed history, care-

need for caution, because tumors of other viscera may simulate hepatomegaly. Special caution is also in order when there is persistent hemorrhage from the skin incision, or if there is a tympanic percussion tone, an indication that there is intestine between the liver and the diaphragm. The tissue specimen should be taken at a depth of 5 5 to 6 5 cm, and firm pressure should be maintained on the site of the biopsy after the intervention. He further recommends 24 hours at bed rest after the procedure, which should be performed in a hospital. Special measures for hemostasis, as suggested by Clay and Dickinson (18), Terry believes are superfluous He agrees with other workers that aspiration biopsy of the liver is practically without danger in young, healthy individuals.

In summary, then, liver biopsy does entail a certain risk, especially of hemorrhage, and this risk cannot be eliminated completely. The fatality rate of this diagnostic procedure varies somewhat, but is around 0.12 per cent. It seems to be the consensus that the magnitude of the risk is not excessive (17, 24, 85, 91) It is essential that anyone making use of this method be thoroughly aware of the risks and the means by which they can be avoided, that cases for biopsy be selected with care, and that the patient be carefully studied and watched before and after the procedure (35).

Efficiency of Method

Exact figures can hardly be given. In one series of 297 biopsies, 59 biopsies (19 9 per cent) yielded either no hepatic tissue, or too little tissue for histologic examination (78). Technical reasons were responsible in 15 of these 59 cases, such as aspiration of blood or bile into the syringe, and patients' restlessness In the remaining 44 cases there was no discernible reason Tyor and Cayer (103) reported that only 2 of 100 biopsies performed with the Vim-Silverman cannula were unsatisfactory, whereas Topp *et al* (100), also using the Vim-Silverman cannula, obtained unsatisfactory specimens in 12 6 per cent of their biopsies.

There is no doubt that the percentage of failures depends on various factors, among them the technic used, the surgeon's experience, and the patient's ability to cooperate. Furthermore, a satisfactory biopsy specimen is more difficult to obtain in certain liver diseases, for example liver cirrhosis, in which the specimen is often irregular and fragmented. The presence of ascites entails special difficulties in ob-

cases of subcutaneous emphysema have been reported, they were without practical significance.

When aspiration biopsy is performed under conditions of strict asepsis, the risk of infection is minimal. It cannot be denied that theoretically an existing infection in the pleura, peritoneum, or liver might be carried by the puncture to other tissues or the blood, but in our experience no such complication has occurred. Nevertheless, biopsy undoubtedly should be avoided in the presence of inflammatory processes in the right lung. Inflammations of the liver and the bile ducts call for circumspection. Biliary peritonitis is a rare complication but one of more than theoretic interest (80, 99) The danger of its occurrence is particularly great in the presence of massive obstruction and considerable dilatation of the bile ducts. One death, possibly due to bile embolism caused by a traumatic communication between a bile duct and the hepatic vein, has been reported (10).

Hemorrhage from the stab wound is by far the most important and dangerous complication.* Small, localized hemorrhages may give rise to a transient pleural or peritoneal reaction, but an intraperitoneal hemorrhage may be fatal In the study of 297 biopsies previously mentioned (78), there were 2 deaths from hemorrhage. Both were cases of cancer, and in both the clotting time of the blood was prolonged.

A recent review of the risks in aspiration biopsy by Terry (99) covers 10,000 biopsies reported in the literature since 1939, there were 13 deaths, and in all the prognosis had been hopeless. He believes that the following precautions must be observed, in order to avoid complications (1) great care to exclude the presence of a hemorrhagic tendency, (2) experience on the part of the surgeon, (3) use of a small-bored cannula, the bore ranging from 1.5 to 1.8 mm That the last is important is borne out by the fact that in 28 biopsies performed by Raby (71) with a cannula of 2 23 mm diameter there were 2 deaths McMichael (60) had 2 deaths in 150 biopsies, he then changed to a cannula of 1 mm. diameter, after which hemorrhages no longer occurred Terry also stresses the importance of effective local anesthesia In certain cases, preparatory duodenal intubation is required, in order to recognize a possibly existing inflammation of the bile ducts.

Terry considers the intercostal route to be the best He urges the

---

*Editors' note —Before a needle biopsy of any parenchymatous organ is performed, bleeding time, clotting time, and prothrombin time should be determined No biopsy should be done if any of these values is abnormal.

to follow, step by step, the relation between acute hepatitis and the subchronic and chronic forms, and also the transition of hepatitis to toxic or coarse nodular cirrhosis (36, 37, 43, 48, 92).

Special attention has been given to the problem whether a high glycogen content of the liver improves the prognosis of hepatitis. Krarup (50) studied 15 patients with acute hepatitis chosen at random and found that even in those with extremely severe cases the parenchymal cells were abundantly supplied with glycogen. Morphologically, the quantities of glycogen in hepatitis did not differ from those

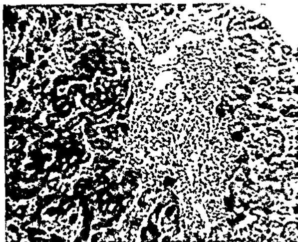


FIG. 2—Liver biopsy specimen in acute hepatitis, Best's carmine stain, ×110

demonstrable in normal liver tissue Apparently, the glycogen disappears within a few hours after death. On the basis of these investigations, the theory upon which the frequently employed treatment with insulin and glucose is based seems questionable (48) Eppinger (26a), too, was of this opinion

Glycogen-stained biopsy preparations from cases of acute hepatitis clearly illustrate the severe inflammatory changes The parenchymal cells are separated by inflammatory tissue showing a varying degree of edema and a varying number of hematic elements (Fig 2).

SUBCHRONIC AND CHRONIC HEPATITIS—The association between acute yellow atrophy of the liver and cirrhosis was demonstrated as early as 1895 by Marchand (61a) Mallory (60a), in 1911, described in detail the histopathologic changes in this form of cirrhosis The view that the

taining a satisfactory specimen. Even with a perfect technic and sufficient experience, a 5 to 10 per cent rate of failures must be reckoned with.

Liver Biopsy in Various Conditions

Acute hepatitis.—Until 1939 the histopathology of hepatitis was scarcely known. Eppinger (26a) examined the livers of 4 soldiers who died of their wounds during an attack of acute hepatitis, and found considerable degenerative changes in the parenchyma. Thereafter,

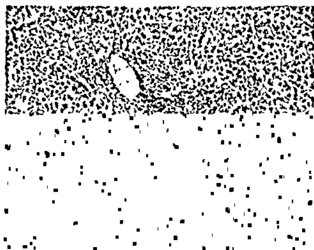


Fig 1.—Liver biopsy specimen in acute hepatitis, hematoxylin-eosin stain, ×120

occasional biopsies performed during surgical procedures revealed similar changes, together with signs of inflammation The results obtained in a series of 38 needle biopsies performed on 26 patients from 3 to 51 days after the onset of jaundice made it possible to follow the evolution of the histopathologic changes which occur in the different forms of hepatitis (42). Thanks to this study, the pathologists have become aware of typical progression of the changes in acute hepatitis, consisting of a severe, diffuse hepatitis with inflammatory phenomena,

varying degree of
orkers, continuing
e of the histopa-
36, 48, 89) and in
other hepatic disorders (33, 37) Aspiration biopsy made it possible

test, galactose test; Takata-Ara test; sedimentation rate; urinary urobilin and urobilinogen. The icterus index and the urinary content of urobilin and urobilinogen were the most sensitive indices of liver damage. The bromsulfalein test in this study was seemingly of no greater diagnostic value than the icterus index.* The results of the galactose test were positive mainly in patients with acute hepatitis,† and even in them the results were doubtful The Takata-Ara test‡ was useful in the diagnosis of chronic hepatitis. In most cases, the sedimentation rate was normal in the first weeks of acute hepatitis, moderately increased in obstructive jaundice, and often increased in cirrhosis

Sherlock (90) found that liver cell damage was most clearly reflected by the serum bilirubin test and by changes in the serum protein values, whereas galactose tolerance was influenced only in the most severe cases A number of workers have studied this problem (63, 66, 67, 70, 72, 112). The consensus is that there is no absolute correlation between liver cell changes and the degree of functional disturbance as revealed by the routine liver function tests Only the alterations in serum protein values apparently parallel the degree of liver cirrhosis Welin (116) has shown that the albumin–globulin ratio is almost invariably changed in cirrhosis and that the slight connective tissue proliferation which develops during the early part of the chronic stage may subside.

The frequent lack of correlation between the results of liver function tests and the histologic changes may in large part be ascribed to the relative crudeness of the ordinary liver function tests. Some have held that the small specimen obtained by aspiration biopsy was at fault, and that the correlation would be better if larger specimens were available We are not of this opinion, however

Steatosis hepatis —To evaluate the frequency of steatosis, a series of 100 consecutive biopsies was studied (41). A varying degree of steatosis was found in 38 of the patients, 28 of whom were alcoholics On the basis of these findings it was concluded that normally there is no fatty infiltration of the liver, and that its presence is tantamount to liver disease. The diagnosis of steatosis hepatis during life can be established with certainty only by means of a biopsy Although

* Editors' note —This is at variance with the experience of most clinicians

† Editors' note —The galactose test is positive mainly in the first weeks of acute hepatitis

‡ Editors' note —In the United States this test has been replaced by the determination of gamma globulin

various forms of hepatitis constitute a nosologic entity gradually gained general acceptance. Practically all reports, however, were based on the findings in autopsy material, and only a few observations were known in which the different phases of the transition of hepatitis into cirrhosis had been studied. In one such study (53), 12 patients with chronic hepatitis were observed by repeated biopsies, and in 6 of them biopsies were performed during the first attack. This investigation showed that the chronic changes may develop rapidly, and that severe alterations may occur even during the first attack (Fig. 3). In the other

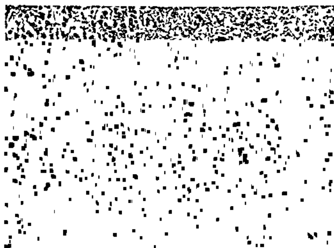


FIG 3 —Liver biopsy in chronic hepatitis, von Gieson-Hansen stain, ×110

6 patients, biopsies were performed a number of times in the course of the chronically recurrent and protracted hepatitis Not one of the patients in this study was addicted to alcohol and the nutritional status of all was excellent The histologic changes which occur during the period when acute hepatitis develops into cirrhosis were elucidated, and they have been confirmed and supplemented by other investigators (65, 107)

Liver biopsy has made clear the correlation which exists between the histopathologic changes of the liver and the results of liver function tests Krarup (51) divided hepatitis into five forms, based on the results of a histopathologic examination of 111 patients: (*1*) acute hepatitis, (*2*) chronic hepatitis, (*3*) steatosis hepatis, (*4*) obstructive jaundice, and (*5*) cancerous hepatitis In each group the following tests were performed at weekly intervals: icterus index, bromsulfalein

jaundice of 1 to 370 days' duration; 16 had calculi in the common bile duct, and 11 had cancer of the bile ducts. Considerable biliary stasis, both within and between the parenchymal cells, was found in all cases (Fig 4). The degenerative changes in the parenchymal cells were more or less pronounced, depending on the duration of the jaundice, but were much less conspicuous than in acute hepatitis Nor were the inflammatory phenomena, which were limited chiefly to the interlobular connective tissue, as distinct as in hepatitis. The characteristic changes of obstructive jaundice appear in an early stage, they can be

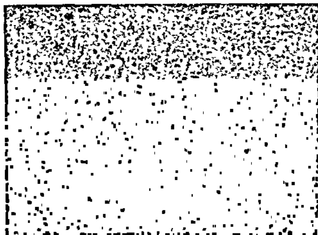


FIG 4.—Liver biopsy specimen in obstructive jaundice, hematoxylin-eosin stain, ×110

recognized within 24 hours, and become pronounced in the course of 3 days. Long-standing obstructive jaundice causes a more conspicuous, irregular fibrosis, often accompanied by rather violent inflammatory phenomena and pronounced parenchymal changes The changes in biliary stasis, particularly in the early stages, present a morphologic picture different from that of hepatitis

JUVENILE INTERMITTENT JAUNDICE.—This term was used by Meulengracht (64) for an apparently definite nosologic entity and which is possibly identical with the "cholémie simple familiale" of Gilbert and Lereboullet (29a), the "physiologische Hyperbilirubinamie" of van den Bergh (106), and the "constitutional hepatic dysfunction" of Rozendaal *et al* (79) Of 5 patients with this form of jaundice studied by Krarup and Roholm (54), the liver was completely normal histologically in 3 and had some degree of fatty infiltration in 2. This

steatosis may possibly be a precursor of cirrhosis, it should not be considered the initial stage of this condition Our observation that steatosis is not an irreversible condition, and that it may subside in the course of weeks or months, has been confirmed by others.

The frequent occurrence of steatosis in alcoholics is generally accepted. In many cases the condition improves or disappears on treatment by adequate diet and rest. Thus Buck (13) found a considerable decrease *of the liver fat content in 5 of 7 alcoholics with fatty* degeneration of the liver after they had been put on an adequate diet Volwiler *et al.* (109) reported that their patients recovered on a regimen of rest and an ordinary mixed diet At the same time, the histologic changes of steatosis disappeared. W [illegible] Yesner (46) obtained a decrease of [illegible] liver cells, and subsidence of inflammatory changes and fibrosis. Sche *et al* (88), who examined 63 alcoholics by repeated aspiration biopsy, stated that the fat content of the liver decreased after a week's hospital stay.

Patients with a severe degree of steatosis may present the clinical picture of liver cirrhosis. This may explain some instances of clinically diagnosed cirrhosis in which a striking improvement was obtained. Biopsy in these cases might have disclosed the presence of steatosis rather than cirrhosis As Ulevitch *et al* (104) have emphasized, biopsy is a valuable diagnostic aid in the differentiation of fatty degeneration and cirrhosis of the liver.

Postarsphenamine jaundice.—The problem of this type of jaundice has been elucidated with the help of liver biopsy Originally, it was believed that syphilitic involvement of the liver was the cause of the jaundice Then, *after the introduction of arsenotherapy,* it was assumed that the jaundice was the result of a toxic reaction to the drug Gradually, however, it became clear that the jaundice was due to homologous serum hepatitis incurred as a result of injection. In 1940, Roholm and Krarup (76) reported the results of liver biopsy in 10 patients with postarsphenamine jaundice, 1 of whom did not have syphilis Inflammatory infiltration, degenerative parenchymal changes, and varying degrees of connective tissue proliferation were *found in all 10. These* lesions were of the same nature and intensity as those that have since been found in homologous serum hepatitis Dible and McMichael (25) reported similar findings.

Obstructive jaundice.—The histopathology of the liver in biliary stasis, as differentiated from that of hepatitis, was studied by Roholm and Krarup (77). Aspiration biopsy was performed in 27 patients with

POSTHEPATITIS SYNDROME.—This syndrome at one time seemed to be of considerable importance, especially in members of the armed forces who had recovered from hepatitis. Patients with this syndrome complained of fatigue and poorly defined gastrointestinal symptoms, but the results of liver function tests were apparently normal. The patients themselves, and their relatives, were often convinced that they were suffering from a chronic liver ailment. Aspiration biopsy in 20 such patients, performed by Sherlock and Walshe (93) revealed completely



FIG. 6—Liver biopsy specimen showing lymphatic leukemic infiltration, ×110

normal liver tissue in all of them These investigators concluded that the patients were suffering from a neurosis

INFECTIOUS MONONUCLEOSIS, BOECK'S SARCOID, LEUKEMIA.—Van Beek and Haex (105), who have performed some 2,000 aspiration biopsies, reported in 1943 that in Boeck's sarcoid there are submiliary, noncaseous, epithelioid tubercles in the liver, this important finding, will in doubtful cases help to establish the diagnosis In 4 patients with infectious mononucleosis complicated by jaundice, Bang and Wanscher (3) found parenchymatous and interstitial inflammatory changes and proliferation in the sinusoids of small, lymphocytic-like cells, presumably belonging to the reticuloendothelial system

Occasionally, liver biopsy will reveal the presence of leukemia (Fig 6) In most cases, the hemorrhagic tendency which is so common in this disease contraindicates liver biopsy.

confirms Meulengracht's hypothesis that juvenile intermittent jaundice is a functional hepatic disorder. Curry *et al.* (21) reported on 1 patient, in whom they were unable to demonstrate any histologic changes in the liver.

HEPATOMEGALY IN DIABETES MELLITUS —The pathologic nature of this well-known condition had not been established; increased fat in the liver, increased storage of glycogen, or increased fluid content had all been considered possible causes. Marble *et al* (61) and White *et al*

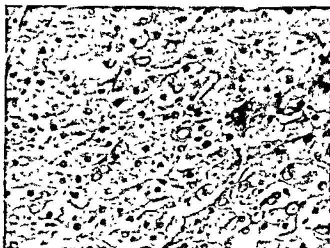


FIG 5—Liver biopsy specimen from patient with diabetes and liver enlargement, note degeneration and glycogen in the nuclei, ×225

(117) concluded that the hepatomegaly was due to an increase of glycogen and a considerable increase in fluid intake, both being the result of insufficient control of the diabetes.

Liver biopsy was performed in 1 of 2 children with diabetes and enlarged liver (52). In both, the diabetes was controlled by diet and insulin, the enlargement of the liver subsided after 50 and 54 days of treatment, respectively. The biopsy before the start of treatment revealed a peculiar enlargement of the individual liver cells, which were of an edematous, clear, meshlike appearance, a moderately severe, fairly diffuse fatty infiltration, consisting chiefly of minute fat globules, was also present. The glycogen content of the liver was not increased, although glycogen was found in some of the nuclei (Fig. 5) The histology of the liver returned to normal, as shown by the biopsy performed after the diabetes was controlled by treatment.

they compared the distribution of lipids, as revealed by the histologic method, with the results obtained by the analytic method, their work was done on autopsy material

The greatest usefulness of liver biopsy is in the diagnosis of hepatic disease which cannot be diagnosed with certainty by other methods Its value when the nature and degree of the hepatic changes must be ascertained, possibly to follow their development or the effect of treatment, is unquestioned. Furthermore, its use may be justified in certain cases to further special investigation

We have found aspiration biopsy to be of special value in the following pathologic conditions of the liver· (*1*) diffuse parenchymal lesions, such as amyloidosis, (*2*) inflammatory or tumor-like chronic infiltration, e.g, Hodgkin's disease, reticulosis, Boeck's sarcoid, leukemia, syphilis, (*3*) acute hepatitis, particularly when the diagnosis is in doubt or when the course must be followed closely, (*4*) chronic hepatitis and cirrhosis, especially when the diagnosis or prognosis is difficult, (*5*) hepatic cancer, when the diagnosis is questionable, (*6*) obstructive jaundice, especially in doubtful cases in which exploratory laparotomy seems indicated, (*7*) juvenile intermittent jaundice, which often must be differentiated from chronic, recurrent hepatitis

KIDNEY BIOPSY

Biopsy of the kidney has been done repeatedly in the course of surgical procedures for arterial hypertension (14) But puncture biopsy of the kidney has been attempted only in recent years. Cazal (15) used it in patients with renal neoplasms, and we have applied the method to the study of the histopathology of medical diseases of the kidney (8, 9, 12, 40) Awakened interest in kidney biopsy led Alwall (1) to report his experience with 10 biopsies performed in 1944

That kidney biopsy, if technically practicable, would afford valuable information was to be expected The procedure could add greatly to our knowledge, insufficient at best, of kidney histology in the early stages of renal disease, as well as of the histologic changes in mild affections of the kidney Such cases rarely come to autopsy, and only serial biopsies of the kidneys will permit the study of the different phases of the disease. Finally, by means of biopsy a histologic picture of the kidney could be obtained which had not suffered any autolytic postmortem process, especially in the tubular cells, such as is no doubt present in most autopsy preparations

Hemochromatosis.—Aspiration biopsy of the liver established the diagnosis in 1 patient with this condition (43).

Pregnancy.—The histology of the liver, both in normal pregnancy and in pregnancies complicated by albuminuria, pre-eclampsia, and eclampsia, was studied by Ingerslev and Teilum (39). Only in a few cases were any histologic changes found; these consisted of precipitations of fibrin, necrosis, and signs of hemorrhage. In most cases, the liver appeared to be completely normal.

Alcoholism —Liver biopsy by Raby (72) in 22 alcoholics revealed normal tissue in only 7 Buck (13), Post and Rose (70), and Davis and Culpepper (22), have also studied the liver in alcoholism by means of biopsy.

Ulcerative colitis —No characteristic changes were found in 32 patients with ulcerative colitis examined by Klechner *et al* (47). Nor could any relation be established between changes in the liver and the duration or severity of the disease

Summary

Use of aspiration biopsy of the liver has increased our knowledge of the histology and histopathology of this organ. The method has been *particularly useful in elucidating the histopathology of the liver* in acute, subacute, and chronic hepatitis, and has led to a much clearer understanding of the disease and its various transition forms, as well as of steatosis hepatitis and its relation to cirrhosis It has contributed to our understanding of the true nature of postarsphenamine jaundice, juvenile intermittent jaundice, the so-called posthepatitis syndrome, and hepatomegaly in diabetes mellitus The various phases of obstructive jaundice have been defined on the basis of their histology, and the histologic changes in the liver in infectious mononucleosis and in toxemia of pregnancy have been investigated

The results of various forms of therapy have been checked by means of the histologic changes as revealed by aspiration biopsy (109, 113). Steigmann (95), for example, followed the effect of lipotropic substances in the treatment of liver cirrhosis by aspiration biopsies

Biochemical study of tissue removed by aspiration biopsy has been possible in several cases (58, 60) A micromethod for the determination of vitamin A in the liver tissue of large animals and man, obtained by biopsy, has been described by With (118). A method of lipid analysis of liver tissue has been elaborated by Billings *et al* (5),

made in the skin and the cannula is introduced until, on the basis of the preceding measurements, it can be assumed that the surface of the kidney has been reached. At this point, the kidney can often be "felt" if the stilet is not too long and sharp. The stilet is then removed, the syringe is attached to the trocar, and the piston is pulled back and fixed in this position, this assures a negative pressure in trocar and syringe. With a screwing movement, the trocar is pushed 2 to 3 cm. into the kidney substance, then rapidly withdrawn, with care being taken to maintain the vacuum in syringe and trocar so long as the latter is within the kidney tissue. The free edge of the trocar exerts a cutting effect during this maneuver, and the cylinder of renal tissue that has been cut out will be caught in the vacuum as trocar and syringe are withdrawn As a rule, the cylinder of tissue will have been aspirated into the syringe. The specimen is at once put into 93 per cent alcohol and placed on a small piece of cardboard. This technic is necessary for staining the alkaline phosphatase with Gomori's method.

Risks

To the authors' knowledge, no fatality has resulted from kidney biopsy. The local pain which is experienced during the procedure is in most cases of short duration. Bleeding as a rule is limited to a transient, microscopic hematuria; only in a few cases have appreciable amounts of blood appeared in the urine In one of Alwall's patients, a 47 year old woman with a nephrotic syndrome, the kidney biopsy was followed by a condition resembling shock, oliguria, anuria, and finally death. However, this patient had only one kidney. In addition, the oliguria had also been observed the day before the biopsy It is impossible to state with certainty whether the death of this patient occurred after the biopsy, or because of the biopsy. In any event, the experience led Alwall to abandon the procedure.

Contraindications.—A hemorrhagic tendency (*see* footnote, p 166), hydronephrosis, and absence of the left kidney are contraindications to kidney biopsy

Normal Renal Histology as Revealed by Biopsy

Histologic study of the normal kidney, by means of biopsy specimens, has been carried out in only a few cases The renal tissue of 2 patients with normal renal function showed a picture different from

Just as aspiration biopsy of the liver has been responsible for improved understanding of liver function in health and disease, kidney biopsy could be instrumental in correlating histology, histochemistry, and function in renal diseases. In this way, knowledge of the pathophysiology, diagnosis, and prognosis of these diseases would be furthered

About 200 kidney biopsies have now been performed with the technic of Iversen and Brun (40), and satisfactory specimens were obtained in about 40 per cent of the cases. Some of the specimens consisted of varying amounts of cortical and medullary tissues, others, only of cortex or of medulla Most of the tissue samples were 5 to 20 mm. long; the longest was 27 mm. In most instances the number of glomeruli varied from 5 to 20, the greatest number observed in a single specimen was 35; and some specimens contained no glomeruli at all The impression gained so far is that these specimens are probably representative samples of tissue in the case of diffuse renal disorders, but hardly in that of focal diseases of the kidney. But this question awaits a detailed statistical analysis such as has already been done for biopsy of the liver.

Definite rules for the use of kidney biopsy cannot yet be established. For the time being, it seems advisable to restrict this method to hospital services with a special interest in its use As additional experience with the technic is accumulated, more refined renal function tests will become possible, leading to a much more detailed study of patients subjected to kidney biopsy. At present, it would seem that the method can be used to best advantage in the acute anurias and the nephrotic syndrome, both for diagnosis and for indications for possible treatment.

Technic

An intravenous pyelogram is made before the biopsy A piece of lead, which serves as a marker, is placed on the patient's skin over the lumbar spine at the level of the right kidney. The exact location of the kidney, in relation to the lead marker, can be determined if the exposures are made in two planes in the sitting position.

The instruments used are the same as those described for liver biopsy. Preparation of the patient consists of disinfection of the skin and application of a local anesthetic. After the patient is placed in the same position as has been used for the pyelogram, a small incision is

nephrosis," it has been pointed out by other workers, is an inappropriate term for this renal disorder, a more appropriate designation would be "acute tubulo-interstitial nephritis" It is noteworthy that in several cases the kidney biopsy revealed a completely normal histology, although there was considerable functional disturbance.

HEPATORENAL SYNDROME.—Kidney biopsy has been performed in 5 *patients with the so-called hepatorenal syndrome*, 2 of them had obstructive jaundice caused by carcinoma of the head of the pancreas,

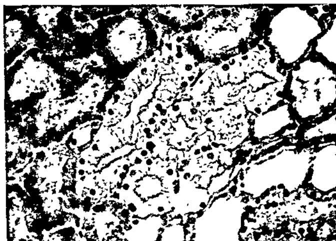


FIG 7.—Kidney biopsy specimen from 26 year old man with crush syndrome on the seventh day of anuria, note hydropic, degenerated proximal convoluted tubules, ×225

1 subchronic hepatitis, 1 liver cirrhosis, and 1 an acute recurrence of chronic cholecystitis Anuria had developed in 4 of the patients, in the fifth, the output of urine could not be estimated A varying degree of uremia was found in all 5 In 1 of the jaundiced patients the biopsy was performed 8 days before the appearance of uremia, in the other 4, the biopsies were done a varying number of days after the appearance of uremia The clinical course and the histologic changes revealed by the biopsy specimen in the patient with chronic cholecystitis resembled acute tubular nephritis or so-called lower nephron nephrosis; the specimens from the other patients revealed no abnormalities apart from bile-stained casts in both distal and collecting tubules

It is hoped that, in time, biopsy may contribute to a better under-

the classic one found in autopsy preparations. Judging from the few specimens examined, the height of the cells in the proximal and distal tubules is ill-defined toward the lumen, and the latter often contain protoplasmic debris. The capillary loops of the glomeruli are generally bloodless, a decided contrast to the usual finding at autopsy. Protein-like precipitates may be present in the capsular spaces, even in the absence of proteinuria. To date, the most important observation is that the normal appearance of the proximal convoluted tubules differs not at all from earlier descriptions of supposedly degenerative tubular changes

Gomori's stain will demonstrate alkaline phosphatase in the normal kidney, present partly in the cells of the luminal part of the proximal convoluted tubules and partly in the ascending portion of Henle's loops.

Renal Histology in Various Disease States

Acute anurias —Kidney biopsies have been performed in 12 patients with acute anuria—10 women and 2 men, ranging in age from 19 to 63 years (*11*) In 6 patients, the acute anuria had developed after sulfonamide therapy, shock had been responsible in 3 patients, it was a complication of acute gastroenteritis in 2, and in 1 patient it followed a febrile abortion Seventeen biopsies were performed in these 12 patients, from 2 to 62 days after onset of the anuria

Histologic examination of the biopsy specimens revealed (*1*) normal glomeruli, (*2*) in the proximal convoluted tubules, a low, flattened epithelium and, in some cases, dilatation of these parts of the tubules, (*3*) dilatation and flattened epithelium of the distal tubules and ascending portion of Henle's loops, (*4*) occasional hydropic changes in the epithelium of the proximal tubules (Fig 7), (*5*) degenerative and regenerative changes in the tubular epithelium as a whole, often present concomitantly, (*6*) focal or diffuse interstitial changes, in the form of edema with or without a varying degree of cellular infiltration, (*7*) hemoglobin-stained casts in the distal convoluted tubules, in Henle's loops, and in the collecting tubules, (*8*) frequently negative, or only faintly positive phosphatase reaction, (*9*) absence of vascular changes

The most important difference between this picture and earlier descriptions of the histology in acute anuria is the prominence of the interstitial changes and the localization of the degenerative changes predominantly in the cells of the proximal tubules "Lower nephron

patients with chronic glomerulonephritis, 3 of the patients had a slight or pronounced uremia, and 2 presented symptoms and signs of a nephrotic syndrome, 1 patient died of uremia. The biopsy specimens revealed the following renal changes. (*1*) glomerular hyalinization of varying degree, combined in some cases with capsular proliferation, and with the presence of normal glomeruli, (*2*) in 1 case, "prehyaline" precipitates in the glomeruli (Fig. 8) which stained yellowish reddish in van Gieson preparations; (*3*) atrophy of the tubules around the glomeruli which had suffered the greatest change, and increased interstitial connective tissue, with inflammatory infiltration, in these areas.



FIG 9 —Kidney biopsy specimen from 12 year old girl with "genuine nephrosis" of 3 months' duration A few dilated distal convoluted tubules, appearance otherwise normal, ×115

The picture, therefore, does not differ from the textbook descriptions.

GENUINE LIPOID NEPHROSIS —The results of kidney biopsy in 2 cases of so-called genuine lipoid nephrosis have been reported (8). In 1 patient, the biopsy specimen consisted exclusively of cortex which contained 20 to 22 glomeruli, mostly normal Only 3 glomeruli were slightly contracted, with a slight hyaline thickening of the basement membranes of the capillary loops. Most of the tubules were normal; a few were atrophic, and some distal tubules were dilated and contained hyaline casts (Fig 9). There were *no vascular changes, nor was* amyloid-staining material found in the tissue. Several biopsies were performed in the other patient The specimens revealed moderate glomerular hyalinization, rather pronounced tubular dilatation, hy-

standing of the hepatorenal syndrome, a condition which still poses many questions to the clinical investigator.

ACUTE AND CHRONIC GLOMERULONEPHRITIS.—Kidney biopsies have been performed in 11 patients with glomerulonephritis—6 with acute cases, 5 with chronic. The patients with acute glomerulonephritis, which followed an acute infection, presented the classic signs of hematuria, albuminuria, edema, and arterial hypertension, 1 patient died of eclampsia and pulmonary edema. Biopsies were performed from 15 to 38 days after the onset of symptoms

The histologic examination revealed (*1*) a slight increase in the

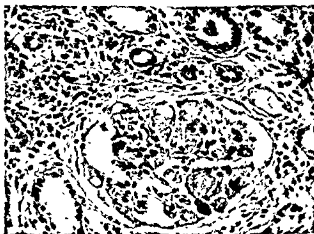


FIG 8—Kidney biopsy specimen from 55 year old woman with nephrotic syndrome of 2 years' duration Chronic glomerulonephritis with "prehyaline" precipitates in the glomerulus, ×225

number of glomerular cells (endothelial and epithelial proliferation), and (*2*) a slight, diffuse hyalinization of the basement membrane In 1 patient, there were more marked changes of a chronic nature several, partly hyaline glomeruli, synechiae, and a single crescent, the latter changes were present in this 1 case only Except for the presence of blood in several of the distal parts, the tubules seemed normal The interstitial changes consisted of a slight edema and inflammatory infiltration On the whole, therefore, the glomerular changes were amazingly scant, much slighter than might have been expected, in view of the clinical picture and the histologic changes of acute glomerulonephritis found on postmortem examination.

Proteinuria of several years' duration had been present in all of the

of the leg, and tertiary syphilis in 1 case each No disease which favors development of secondary amyloidosis could be discovered in the history of 1 patient, and the case was therefore considered to be one of so-called primary amyloidosis.

DIABETIC NEPHROPATHY —Results of biopsies in 12 patients with diabetes mellitus—9 women and 3 men, chiefly in the older age groups —have been reported (12). The diabetes was mild in all but 1 patient. Retinopathy, arterial hyertension, and bacilluria were present in most of them. The biopsy specimens disclosed diffuse (7 cases) or nodular and diffuse (4 cases) intercapillary glomerulosclerosis in 11 cases, and a normal kidney in the twelfth patient. The renal manifestations in this last patient were ultimately found to be due to heart failure, and they cleared up when treatment improved the heart condition. No microaneurysms in the glomeruli were seen. Other changes found in the renal tissues were tubular atrophy and an increase of interstitial connective tissue, it was felt that the degree of these changes might correspond to the number of involved glomeruli. The vascular changes that were present also approximately corresponded to the intensity of the glomerular lesions The decreased phosphatase reaction corresponded to the tubular atrophy Inflammatory infiltration, suggesting the presence of chronic pyelonephritis, was found in 1 patient only, although 8 had bacilluria and pyuria

In this series of patients with diabetic nephropathy, as in other reported series, the nodular glomerular change was found chiefly in the elderly women whose diabetes was of long standing and comparatively mild (Fig 11) The slight, diffuse changes, on the other hand, are usually present in patients who have had diabetes for a relatively short time Nevertheless, in 2 patients with long-standing diabetes, severe diffuse changes without nodules were found The results of this study seem to support the theory that nodular changes in the glomeruli develop from the diffuse form of renal disease

RENAL CALCINOSIS —The results of kidney biopsy in 2 cases of renal calcinosis have been reported (9) One was a case of hyperparathyroidism, the other of calciferol (vitamin D_2) intoxication. The biopsy specimen from the hyperparathyroid patient revealed considerable intracanalicular and interstitial deposits of calcium in cortex and medulla, as well as interstitial fibrosis and numerous hyalinized glomeruli The biopsy specimen from the patient with calciferol intoxication, taken immediately on hospitalization, consisted chiefly of medullary tissue containing large calcific deposits apparently localized

dropic (or lipoid) degeneration of some tubular cells, and a slight, diffuse increase of the interstitial connective tissue. Presumably, this case represents a transitional form between chronic glomerulonephritis and genuine nephrosis. After ACTH therapy had produced marked improvement of the nephrotic syndrome, with almost complete disappearance of the proteinuria and edema, biopsy revealed an essentially unchanged histologic picture, the only difference being increased height of the cells in the proximal tubules and a more pronounced cellular phosphatase reaction

RENAL AMYLOIDOSIS.—Six cases of renal amyloidosis have been



FIG. 10—Kidney biopsy specimen from 38 year old man with chronic polyarthritis and nephrotic syndrome of 6 years' duration Note total amyloid degeneration of glomerulus, ×225

diagnosed by means of kidney biopsy. The clinical picture in all of the patients was characterized by proteinuria, which in 4 patients was considerable. Edema was present in 5 patients, in some it was pronounced, and was combined with a marked decrease of the serum albumin concentration None had hypertension Uremia was the cause of death in 4 patients

Specific stains demonstrated the presence of typical amyloid changes, especially in the glomeruli In most cases, the changes were severe, and such as are usually found in the amyloid-contracted kidney (Fig 10).

Chronic bronchitis and bronchiectasis caused the amyloidosis in 2 cases, rheumatoid arthritis, in 2 cases, and pneumonia, chronic ulcer

which have the same symptomatology but widely different etiology and clinical course, seems to be possible only by the use of kidney biopsy. It is particularly noteworthy that the diagnosis of renal amyloidosis could, in some patients, be established only by means of a biopsy. The condition has been diagnosed by this method in 6 patients; only in 2 had a presumptive diagnosis of secondary amyloidosis been made—in a patient with tertiary syphilis and in another with a chronic ulcer of the leg. The clinical diagnosis in the other 4 patients had been chronic nephropathy of unspecific type, chronic glomerulonephritis, acute exacerbation of chronic glomerulonephritis, and Sanocrysin intoxication.

Kidney biopsies in 12 patients with diabetes mellitus have demonstrated the value of this diagnostic method in diabetic nephropathy. In 1 case the biopsy was instrumental in correcting a mistaken diagnosis of nephropathy, for the patient was actually suffering from heart failure. In some cases in which the biopsy merely confirmed the clinical diagnosis, it nevertheless provided information about the nature of the glomerular changes. This may possibly be of prognostic significance. In others, particularly in those without proteinuria, the biopsy gave the clue to incipient diabetic renal disease which could not yet be diagnosed clinically.

From the limited experience with kidney biopsy in renal calcinosis, it would seem that in this condition, too, the method may prove of value in the prognosis. For example, in a patient with hyperparathyroidism, kidney biopsy revealed severe anatomic changes in the kidney, and the prognosis was therefore believed to be poor; follow-up of this patient showed a further reduction of renal function Follow-up of a patient with calciferol intoxication, on the other hand, in whom the biopsy had shown only slight anatomic changes, revealed that renal function had improved considerably

Summary

Like biopsy of the liver, kidney biopsy is a valuable procedure because it permits a more accurate diagnosis of the nature of various renal disorders This is particularly important in conditions which present the same clinical syndrome but have widely different anatomic changes In such cases, when purely clinical examination is supplemented by biopsy study, the differential diagnosis is markedly advanced

mainly within the tubular lumens; the connective tissue was slightly increased, and there was a slight, diffuse interstitial infiltration by lymphocytes and histiocytes. A second biopsy specimen, obtained 3 weeks after vitamin administration had been discontinued, consisted

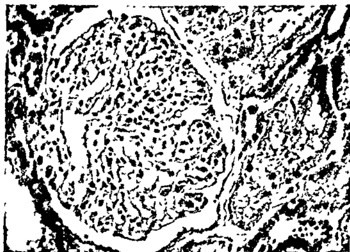


FIG 11 – Kidney biopsy specimen from 65 year old woman with diabetes of 20 years' duration Diffuse and mild nodular Kimmelstiel-Wilson lesion; ×225

of cortical tissue, it revealed nothing definitely abnormal, and calcific deposits were no longer found.

In both cases, the histologic findings were in accord with those of reported postmortem examinations

As Diagnostic Method

At the moment, the practical value of kidney biopsy cannot be assessed accurately, for the method is too new. Experience so far, however, seems to indicate that it can contribute considerably to the diagnosis and prognosis of renal disease.

The diagnostic value of kidney biopsy has been proved by the results obtained in patients with a nephrotic syndrome and with diabetic nephropathy Of 8 patients with the nephrotic syndrome, all of whom presented the characteristic signs, including proteinuria, edema, lowered serum albumin and increased serum cholesterol values, 4 were shown to have renal amyloidosis, 2 chronic glomerulonephritis, and 2 genuine nephrosis Differential diagnosis of diseases such as these,

which have the same symptomatology but widely different etiology and clinical course, seems to be possible only by the use of kidney biopsy. It is particularly noteworthy that the diagnosis of renal amyloidosis could, in some patients, be established only by means of a biopsy. The condition has been diagnosed by this method in 6 patients, only in 2 had a presumptive diagnosis of secondary amyloidosis been made—in a patient with tertiary syphilis and in another with a chronic ulcer of the leg The clinical diagnosis in the other 4 patients had been chronic nephropathy of unspecific type, chronic glomerulonephritis, acute exacerbation of chronic glomerulonephritis, and Sanocrysin intoxication.

Kidney biopsies in 12 patients with diabetes mellitus have demonstrated the value of this diagnostic method in diabetic nephropathy. In 1 case the biopsy was instrumental in correcting a mistaken diagnosis of nephropathy, for the patient was actually suffering from heart failure In some cases in which the biopsy merely confirmed the clinical diagnosis, it nevertheless provided information about the nature of the glomerular changes. This may possibly be of prognostic significance In others, particularly in those without proteinuria, the biopsy gave the clue to incipient diabetic renal disease which could not yet be diagnosed clinically.

From the limited experience with kidney biopsy in renal calcinosis, it would seem that in this condition, too, the method may prove of value in the prognosis. For example, in a patient with hyperparathyroidism, kidney biopsy revealed severe anatomic changes in the kidney, and the prognosis was therefore believed to be poor, follow-up of this patient showed a further reduction of renal function Follow-up of a patient with calciferol intoxication, on the other hand, in whom the biopsy had shown only slight anatomic changes, revealed that renal function had improved considerably

Summary

Like biopsy of the liver, kidney biopsy is a valuable procedure because it permits a more accurate diagnosis of the nature of various renal disorders This is particularly important in conditions which present the same clinical syndrome but have widely different anatomic changes. In such cases, when purely clinical examination is supplemented by biopsy study, the differential diagnosis is markedly advanced

mainly within the tubular lumens; the connective tissue was slightly increased, and there was a slight, diffuse interstitial infiltration by lymphocytes and histiocytes A second biopsy specimen, obtained 3 weeks after vitamin administration had been discontinued, consisted

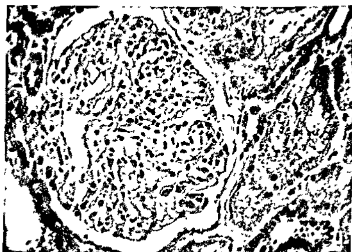


FIG 11 —Kidney biopsy specimen from 65 year old woman with diabetes of 20 years' duration Diffuse and mild nodular Kimmelstiel-Wilson lesion, ×225

of cortical tissue, it revealed nothing definitely abnormal, and calcific deposits were no longer found

In both cases, the histologic findings were in accord with those of reported postmortem examinations

As Diagnostic Method

At the moment, the practical value of kidney biopsy cannot be assessed accurately, for the method is too new Experience so far, however, seems to indicate that it can contribute considerably to the diagnosis and prognosis of renal disease

The diagnostic value of kidney biopsy has been proved by the results obtained in patients with a nephrotic syndrome and with diabetic nephropathy Of 8 patients with the nephrotic syndrome, all of whom presented the characteristic signs, including proteinuria, edema, lowered serum albumin and increased serum cholesterol values, 4 were shown to have renal amyloidosis, 2 chronic glomerulonephritis, and 2 genuine nephrosis Differential diagnosis of diseases such as these,

5 Billings, B. H, *et al* · The value of needle biopsy in the chemical estimation of liver lipids in man, J Lab & Clin Med 40 780, 1952
6 Bingel, A Über die Parenchympunktion der Leber, Verhandl deutsch Gesellsch inn Med 35.210, 1923
7 Bjørneboe, M, Iversen, P, and Keiding, R. The importance of liver biopsy in differential diagnosis, especially in connection with operation, Acta med scandinav. 135 303, 1949
8 Bjørneboe, M, *et al* The nephrotic syndrome I Histological changes illustrated by means of biopsy of the kidney, Acta med scandinav, supp 266.283, 1952
9 Bjørneboe, M, *et al* Two cases of calcinosis renis, studied by means of renal biopsy and renal function tests, J Clin Invest 31 727, 1952
10 Brown, C Y, and Walsh, G C Fatal bile embolism following liver biopsy, Ann Int Med 36 1529, 1952
11 Brun, C Acute Anuria (Thesis [Copenhagen Munksgaard Forlag, 1954])
12 Brun, C, *et al* · Diabetic nephropathy Kidney biopsy and renal function tests, Am J Med 15:187, 1953
13 Buck, R E · Observations on alcoholic fatty liver The use of interval needle biopsy and liver function tests, J Lab & Clin Med 33.555, 1948
14 Castleman, B, and Smithwick, R H The relation of vascular disease to *the hypertensive state based on the study of renal biopsies from 100 hypertensive* patients, J A M A 121 1256, 1943
15 Cazal, P La place des ponctions-biopsies du foie en hépatologie et en médecine générale, Presse méd 57 480, 1949
16 Chiray, M, Fiessinger, N, and Roux, M La ponction-biopsie du foie, Presse méd 49 785, 1941
17 Christian, E R An evaluation of needle biopsy of the liver, Am J Med 13 689, 1952
18 Clay, H L, and Dickinson, L Needle biopsy of the liver using oxidized cellulose and thrombin to prevent hemorrhage, J Lab & Clin Med 34 422, 1949
19 Cogswell, R C, *et al* Needle biopsy of the liver, J A M A 140 385, 1949
20 Cohen, A G, and Giges, B Punch biopsy of liver in detection hematogenous tuberculosis, J A M A 146 1416, 1951
21 Curry, J J, Greenwalt, T J and Tat, R J Familial nonhemolytic jaundice Report of a case with liver biopsy, New England J Med 226 909 1942
22 Davis, W D, Jr, and Culpepper, W S Cirrhosis of the liver associated with alcoholism Report of acute exacerbation with serial liver biopsies, Ann Int Med 29 942, 1948
23 Davis, W S, Scott, R W, and Lund, H Z Needle biopsy of the liver, Am J M Sc, 212 449, 1946
24 Deschamps, S H, and Steer, A Experience with needle liver biopsies at the Hepatitis Center for Japan and Korea, 1950–51, Am J Med 13 674, 1952
25 Dible, J H, and McMichael, J The pathology of arseno-therapy jaundice, Brit J Ven Dis 19.102, 1943
26 Dible, J. H, McMichael, J, and Sherlock, S P V Pathology of acute

There is no doubt that biopsy will permit an especially fruitful study of the relation between renal anatomy and function. The refinement of renal function tests in general, and the quantitative aspects of these functions in particular, give every reason to expect even better results from biopsy of the kidney than have been obtained with liver biopsy.

The few observations already made seem to point in this direction. For example, in acute anuria there may be considerable renal functional disturbance without appreciable histologic changes: biopsy has revealed the presence of completely normal glomeruli despite a considerably decreased glomerular filtration rate. In diabetic nephropathy, the extent of the renal lesions could by and large be correlated with the presence or absence of proteinuria, and a certain parallelism seemed to exist between renal function and the histologic lesions as revealed by the biopsy. However, in several cases the glomerular filtration rate was relatively well preserved, despite widespread glomerular changes

We believe that when larger series have been studied by this method, important data will emerge on the relation between function and histology. Study of biopsy tissue with the electron microscope may possibly reveal subtle changes in structure

Another field in which kidney biopsy may prove of great value is in the study of enzymic activity in renal tissue Although biopsy specimens of renal tissue have been examined for the presence of alkaline phosphatase by Gomori's method, the final evaluation of these results will be possible only when recent criticisms (44) have been clarified.

Finally, it is hoped that microchemical methods may be developed for the study of tissue and applied to the study of renal tissue obtained by biopsy

REFERENCES

1 Alwall, N Aspiration biopsy of the kidney, including report of a case of amyloidosis diagnosed through aspiration biopsy of the kidney in 1944 and investigated at an autopsy in 1950, Acta med scandinav 143 430, 1952

2 Axenfeld, H , and Brass, K Klinische und bioptische Untersuchungen über den sogenannten Icterus catarrhalis, Frankfurt Ztschr Path. 57 147, 1942

3 Bang, J , and Wanscher, O The histopathology of the liver in infectious mononucleosis complicated by jaundice, investigated by aspiration biopsy, Acta med scandinav 120 437, 1945

4 Baron, E Aspiration for removal of biopsy material from the liver Report of 35 cases, Arch Int Med 63 276, 1939

rest, alcohol withdrawal, and a minimal basic diet, J Clin Invest 28 723, 1949

47. Kleckner, M S, Jr, *et al* Hepatic lesions in the living patient with chronic ulcerative colitis as demonstrated by needle biopsy, Gastroenterology 22:13, 1952
48 Kofler, W Die Leberpunktion als brauchbare und wertvolle klinische Untersuchungsmethode, Ztschr. klin. Med 138·744, 1940.
49 Koszalka, M. F., *et al* · Hepatitis and its sequelae, including the development of portal cirrhosis, Arch Int Med 84 782, 1949.
50 Krarup, N. B. Histological examination of liver glycogen especially in hepatitis epidemica, Acta path et microbiol. scandinav 16 443, 1939
51 Krarup, N B · Über die Relation von einigen Leberfunktionsproben zu der mittels Aspirationsbiopsie untersuchten Histopathologie der Leber, Klin. Wchnschr 22.401, 1943
52 Krarup, N B, and Iversen, P: Enlargement of the liver in diabetes mellitus Examination with liver biopsy, Acta med. scandinav. 105 443, 1941.
53. Krarup, N B, and Roholm, K.· The development of cirrhosis of the liver after acute hepatitis, elucidated by aspiration biopsy, Acta med. scandinav. 108 306, 1941
54 Krarup, N B, and Roholm, K. Leberbiopsie bei Icterus intermittens juvenilis Histologische Untersuchungen, klin. Wchnschr. 20.193, 1941, and Ugesk. laeger 103 72, 1941
55 Kumpe, C. W., *et al* Needle biopsy of the liver: I. General considerations, Gastroenterology 9 672, 1947
56 Lichtman, S S *Diseases of the Liver, Gallbladder and Bile Ducts* (2d ed, Philadelphia Lea & Febiger, 1949).
57. Lucatello Cited by Schupfer (87)
58 Macavy, J Recherches Histochimique et Histophysiologiques sur le Foie Humain Normal et Pathologique Prelevé par Ponction-biopsie, 1952 (Montpelier Dissertation)
59 McHardy, G, Browne, D C, and Edwards, E Peritoneoscopic and biopsy evaluation of hepatic disease, Gastroenterology 9 682, 1947
60 McMichael, J Disease of the liver A review of some clinical and biochemical problems as revealed by systematic biopsy studies, J A M A 137 234, 1948
60a Mallory, F B Cirrhosis of the liver Five different types of lesions from which it may arise, Johns Hopkins Hosp Bull 22 69, 1911.
61 Marble, A, *et al* Enlargement of the liver in diabetic children I. Its incidence, etiology and nature, Arch Int Med 62 740, 1938.
61a Marchand, F Über Ausgang der acuten Leberatrophie in multiple knotige Hyperplasie, Beitr path Anat u allg Path 17.206, 1895
62 Marshall, D Liver biopsy complicated by occult intraabdominal bleeding leading to death Report of a case, Gastroenterology 22 119, 1952.
63. Mateer, J G, *et al* Combined liver biopsy and liver function study in 132 cases of cholelithiasis and 31 cases of peptic ulcer (operated cases), Gastroenterology 11 284, 1948
64 Meulengracht, E Icterus intermittens juvenilis, Klin Wchnschr 18 118, 1939.

hepatitis Aspiration biopsy studies of epidemic, arsenotherapy and serum jaundice, Lancet 2 402, 1943

26a. Eppinger, H *Leberkrankheiten, allgemeine und spezielle Pathologie und Therapie der Leber* (Vienna: Julius Springer Verlag, 1937).

27. Flood, C A, and James, E. M: Clinical and pathological findings in prolonged hepatitis, Gastroenterology 8.175, 1947.

28. Franseen, C C Aspiration biopsy, with a description of a new type of needle, New England J Med 224 1054, 1941

29. Gertz, T. C · Om Sikkerheden af diagnosen ved Leveraspirationsbiopsi [On making certain of the diagnosis by aspiration of the liver], Ugesk laeger 105.34, 1943

29a Gilbert, A, and Lereboullet, P La cholémie simple familiale, Gaz hebd méd 49 889, 1902

30 Gillman, T Discussion of Steigmann (95).

31 Gillman, T, and Gillman, J A modified liver aspiration biopsy apparatus and technique, with special reference to its clinical applications as assessed by 500 biopsies, South African J M Sc 10 53, 1945

32 Gomori, G The distribution of phosphatase in normal organs and tissues, J Cell & Comp Physiol 17 71, 1941

33 Greene, C H Diseases of the liver and biliary tract Hepatic involvement in various diseases related to the war, Arch Int Med 73 349, 1944

34 Hatieganu, I, *et al* Der Wert der Leberpunktion in der Diagnose der verschiedenen Lebererkrankungen, Wien klin Wchnschr 56 21, 1943

35 Hemorrhage from puncture biopsy of the liver, J A M A 129 680, 1945 (Current Comment)

36 Hoffbauer, F. W Needle biopsy of liver, J A M A 134 666, 1947

37 Hoffbauer, F W, Evans, G T, and Watson, C J Cirrhosis of the liver, with particular reference to correlation of composite liver function studies with liver biopsy, M Clin North America, 29 363, 1945

38 Hoffman, J, and Rosenthal, J The liver biopsy An analysis of pathological findings in 75 cases, Ann Int Med 33 1203, 1950

39 Ingerslev, M, and Teilum, G Biopsy studies on the liver in pregnancy, Acta obst et gynec scandinav 25 339, 352, and 361, 1945

40 Iversen, P, and Brun, C Aspiration biopsy of the kidney, Am J Med 11 324, 1951

41 Iversen, P, and Krarup, N B Steatosis hepatis treated with vitamin B_1 Examination with liver biopsy, Acta med scandinav 105 441, 1940

42 Iversen, P, and Roholm, K On aspiration biopsy of the liver, with remarks on its diagnostic significance, Acta med scandinav 102 1, 1939

43 Iversen, P, and Warming-Larsen, A Case of hemochromatosis diagnosed by liver biopsy, Acta med scandinav (supp) 206 344, 1946.

44 Johansen, G, and Linderstrøm-Lang, K Liberation, diffusion, and precipitation of phosphate in the Gomori test, Acta med scandinav (supp) 266 601, 1952

45. Keil, P. G., *et al* Evaluation of the liver biopsy, Ann Int Med 36 1278, 1952

46 Klatskin, G, and Yesner, R Factors in the treatment of Laennec's cirrhosis: I, Clinical and histological changes observed during a control period of bed-

rest, alcohol withdrawal, and a minimal basic diet, J Clin. Invest. 28 723, 1949.

47. Kleckner, M S, Jr., *et al* · Hepatic lesions in the living patient with chronic ulcerative colitis as demonstrated by needle biopsy, Gastroenterology 22·13, 1952

48 Kofler, W Die Leberpunktion als brauchbare und wertvolle klinische Untersuchungsmethode, Ztschr Klin Med. 138.744, 1940

49 Koszalka, M F, *et al*: Hepatitis and its sequelae, including the development of portal cirrhosis, Arch Int. Med 84 782, 1949

50 Krarup, N. B Histological examination of liver glycogen especially in hepatitis epidemica, Acta path et microbiol scandinav. 16 443, 1939.

51 Krarup, N. B Über die Relation von einigen Leberfunktionsproben zu der mittels Aspirationsbiopsie untersuchten Histopathologie der Leber, Klin Wchnschr 22 401, 1943

52 Krarup, N B, and Iversen, P · Enlargement of the liver in diabetes mellitus: Examination with liver biopsy, Acta med scandinav 105 443, 1941.

53 Krarup, N B, and Roholm, K The development of cirrhosis of the liver after acute hepatitis, elucidated by aspiration biopsy, Acta med scandinav. 108 306, 1941

54 Krarup, N B, and Roholm, K Leberbiopsie bei Icterus intermittens juvenilis. Histologische Untersuchungen, klin Wchnschr. 20 193, 1941, and Ugesk laeger 103 72, 1941

55 Kumpe, C W, *et al* Needle biopsy of the liver: I General considerations, Gastroenterology 9 672, 1947.

56 Lichtman, S S *Diseases of the Liver, Gallbladder and Bile Ducts* (2d ed, Philadelphia Lea & Febiger, 1949)

57 Lucatello Cited by Schupfer (87).

58 Macavy, J Recherches Histochimique et Histophysiologiques sur le Foie Humain Normal et Pathologique Prélevé par Ponction-biopsie, 1952 (Montpelier Dissertation)

59 McHardy, G, Browne, D C, and Edwards, E Peritoneoscopic and biopsy evaluation of hepatic disease, Gastroenterology 9 682, 1947

60 McMichael, J Disease of the liver A review of some clinical and biochemical problems as revealed by systematic biopsy studies, J A M A 137 234, 1948

60a Mallory, F B. Cirrhosis of the liver Five different types of lesions from which it may arise, Johns Hopkins Hosp Bull 22 69, 1911

61 Marble, A, *et al* Enlargement of the liver in diabetic children I Its incidence, etiology and nature, Arch Int Med 62 740, 1938

61a Marchand, F Über Ausgang der acuten Leberatrophie in multiple knotige Hyperplasie, Beitr path Anat u allg Path 17.206, 1895

62 Marshall, D Liver biopsy complicated by occult intraabdominal bleeding leading to death Report of a case, Gastroenterology 22 119, 1952

63 Mateer, J G, *et al* Combined liver biopsy and liver function study in 132 cases of cholelithiasis and 31 cases of peptic ulcer (operated cases), Gastroenterology 11 284, 1948

64 Meulengracht, E Icterus intermittens juvenilis, Klin Wchnschr. 18 118, 1939

65. Movitt, E. R · *Jaundice: Its Pathogenesis and Differential Diagnosis* (New York· Oxford University Press, 1947).

66. Moyer, J. H , and Wurl, O A.: Liver biopsy· Correlation with clinical and biochemical observations, Am J. M Sc 221·28, 1951.

67 Norcross, J W., *et al* Liver function An attempt to correlate structural change with functional abnormality, Ann. Int Med. 35 1110, 1951.

68 *Olivet, J.· Die diagnostische Leberparenchympunktion (Lpp), Med. Klin* 22:1440, 1926

69. Popper, H., and Franklin, M · Diagnosis of hepatitis by histologic and functional laboratory methods, J A M A. *131 230, 1948.*

70 Post, J , and Rose, J V Clinical, functional and histologic studies in Laënnec's cirrhosis of the liver, *Am. J Med 8 300, 1950.*

71. Raby, K · Om Komplikationer og farer ved leverbiopsi [Complications and dangers of liver biopsy], Nord. med (Hospitalstid) *24 2161, 1944.*

72 Raby, K Nogle tilfaelde af leverbiopsi ved chronisk alkoholisme [Liver biopsy in chronic alcoholism. *Preliminary report*], Nord med (Hospitalstid) 24 2170, 1944.

73 Ricketts, W E , *et al* · Pathologic observations in portal cirrhosis, *Gastro*enterology 14 491, 1950.

74. Roberts, H , and Hess, H . Ein neues Gerat zur gezielten Leberpunktion, Deutsche med Wchnschr 76 248, 1951

75 Roholm, K , and Iversen, P Changes in the liver in acute epidemic hepatitis *(catarrhal jaundice) based on 38 aspiration biopsies, Acta path et micro*biol scandinav 16 427, 1939

76 Roholm, K , and Krarup, N B Die Histopathologie der Leber bei sog Salvarsanikterus, mittels Aspirationsbiopsie untersucht, Arch Dermat u Syph 181 521, 1940, and Ugesk laeger 103 68, 1941

77. Roholm, K , and Krarup, N B Histopathology *of the liver in obstructive jaundice examined by aspiration biopsy, Acta med scandinav 108 48, 1941*

78 Roholm, K , Krarup, N B , and Iversen, P Aspirationsbiopsie der Leber Mit einer Übersicht uber die Ergebnisse bei 297 Biopsien, Ergebn inn Med u Kinderh 61 635, 1942

79 Rozendaal, H M , Comfort, M W , and Snell, A M Slight and latent jaundice *The significance of elevated concentrations of bilirubin giving an* indirect van den Bergh reaction, J A M A 104.374, 1935

80. Rubenstone, A I , Mintz, S S , and Meranze, D R A case of fatal bile peritonitis following liver needle biopsy, Ann Int Med 36 166, 1952

81 Rumball, J M , and Baum, G L Liver biopsy culture in the diagnosis of miliary tuberculosis A case report, Gastroenterology 22 124, 1952

82 Safdi, S A , *et al* Needle biopsy of the liver II Experiences with malignant neoplasm, Gastroenterology 11 93, 1948

83 Sborov, V. M Simple guard for Vim-Silverman needle, *J Lab & Clin* Med 36·773, 1950

84. Scalabrino, R La biopsia degli organi interni, Arch per le sc. med *53 273,* 1928

85 Schiff, L The clinical value of needle biopsy of the liver, Ann Int Med 34 948, 1951

86 Schiff, L.: *Differential Diagnosis of Jaundice* (Chicago· Year Book Publishers, Inc, 1946)
87 Schupfer, F. De la possibilité de faire intra vitam un diagnostic histo-pathologique précis des maladies du foie et de la rate, Semaine méd. 27.229, 1907.
88 Seife, M; Kessler, B. J, and Lisa, J. R: Clinical, functional and needle biopsy study of the liver in alcoholism, Arch. Int. Med 86 658, 1950.
89 Sherlock, S.. Aspiration liver biopsy Technique and diagnostic application, Lancet 2 397, 1945.
90 Sherlock, S. Biochemical investigations in liver disease Some correlations with hepatic histology, J. Path & Bact 58 523, 1946
91. Sherlock, S: Discussion of Cogswell *et al.* (19).
92. Sherlock, S. Post-hepatitis cirrhosis, Lancet 1.817, 1948
93 Sherlock, S, and Walshe, V The post-hepatitis syndrome, Lancet 2 482, 1946
94 Sommerfelt, S C Malignt hepatom med implantasjonsmetastase etter lever-punksjon [Malignant hepatoma with implantation following liver puncture], Nord med 46 1492, 1951
95. Steigmann, F. Efficacy of lipotropic substances in treatment of cirrhosis of liver, J A M A 137 239, 1948.
96. Steigmann, F, Popper, H, and Meyer, K A Liver function tests in clinical medicine, J A M A 122 279, 1943
97. Svaar-Seljesaeter, O Leverpunksjon som diagnostisk metode [Liver biopsy as diagnostic method], Tidsskr norske laegefor 65 407, 1945
98 Terry, R Needle biopsy of the liver, with special reference to a modified Gillman technique, Brit M J 1 657, 1949
99 Terry, R Risks of needle biopsy of the liver, Brit M. J 1 1102, 1952
100 Topp, J H, Lindert, M C F, and Murphy, F D Needle biopsy of the liver, Arch Int Med 81 832, 1948
101 Tripoli, C J, and Fader, D E The differential diagnosis of certain diseases of the liver by means of punch biopsy, Am J Clin Path 11 516, 1941
102 Tumen, H J Diagnostic Laboratory Methods Applicable to the Study of Liver Function and Disease, in Bockus, H L (ed) *Gastro-enterology* (Philadelphia W B Saunders Company, 1946), Vol 3, pp 62–98
103 Tyor, M P, and Cayer, D The clinical and experimental value of needle biopsy of the liver, Gastroenterology 21 245, 1952
104 Ulevitch, H, *et al* Needle biopsy of the liver VII Observations in fatty vacuolization of the liver, Gastroenterology 18 1, 1951
105 van Beek, C, and Haex, A J C Aspiration-biopsy of the liver in mononucleosis infectiosa and Besnier-Boeck-Schaumann's disease, Acta med scandinav 113 125, 1943
106 van den Bergh, A A H *Der Gallenfarbstoff im Blute* (Leipzig J H Barth, 1918)
107 Volwiler, W, and Elliott, J A, Jr Late manifestations of epidemic infectious hepatitis, Gastroenterology 10 349, 1948
108 Volwiler, W, and Jones, C M The diagnostic and therapeutic value of liver biopsies, with particular reference to trocar biopsy, New England J Med 237 651, 1947

109 Volwiler, W.; Jones, C M., and Mallory, T. B · Criteria for the measurement of results of treatment in fatty cirrhosis, Gastroenterology 11:164, 1948.
110 Waldenstrom, H.· On the formation and disappearance of amyloid in man, Acta chir. scandinav. 63 479, 1928.
111. Waldstein, S S, and Szanto, P. B.: Accuracy of sampling by needle biopsy in diffuse liver disease, Arch Path 50 326, 1950
112. Waldstein, S S, *et al.*: Liver cirrhosis Relation between function and structure based on biopsy studies, A M A. Arch Int Med 87:844, 1951.
113. Waterlow, J. C. *Fatty Liver Disease in Infants in the British West Indies*, Medical Research Council Special Report Series no 263 (London H M Stationery Office, 1948).
114. Watson, C J, and Hoffbauer, F. W. The problem of prolonged hepatitis with particular reference to the cholangiolitic type and to the development of cholangiolitic cirrhosis of the liver, Ann Int. Med 25.195, 1946
115 Weisbrod, F G, *et al* Needle biopsy of the liver III Experiences in the differential diagnosis of jaundice, Gastroenterology 14.56, 1950.
116. Welin, G *Needle Biopsy and Liver Function in Acute Hepatitis and Cirrhosis of the Liver*, 1952 (Goteburg, Dissertation)
117 White, P, *et al* Enlargement of the liver in diabetic children. II Effect of raw pancreas, betaine hydrochloride and protamine insulin, Arch Int Med 62 751, 1938
118 With, T K · Micro-method for the determination of vitamin A in liver biopsies in man and larger animals, Biochem. J 40 249, 1946

Thrombotic Thrombocytopenic Purpura*

KARL SINGER

Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital, Chicago

THERE IS general agreement that thrombocytopenic purpura is not a disease entity but represents rather a syndrome of varied etiology. It may occur as the idiopathic (Werlhof) type, or it may be associated with a great variety of infectious, granulomatous, neoplastic, toxic, or allergic disorders. A special variant of this syndrome, often designated thrombotic thrombocytopenic purpura (96), is being recognized with increasing frequency (Fig. 1).

The outstanding feature of the last-mentioned condition is the appearance of innumerable occlusions of small-caliber vessels in many organs of the body. In typical instances, the clinical manifestations of the disease, in addition to the thrombocytopenic purpura, are the simultaneous existence of a severe hemolytic anemia and transitory, bizarre neurologic symptoms and signs. This symptomatologic triad is so characteristic that the disorder can be readily diagnosed *in vivo*, provided the observer is familiar with this clinical picture. Since thrombotic purpura can no longer be regarded as a mere medical curiosity but must always be considered in the differential diagnosis of a thrombocytopenic syndrome, a review of the available knowledge about this entity may be of general interest.

---

* The author wishes to express his indebtedness to Dr. Margaret Littman, Assistant Pathologist, Michael Reese Hospital, for preparing the histologic slides.

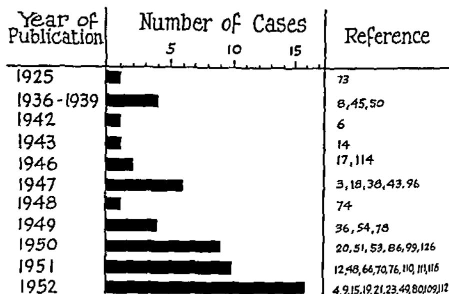


FIG. 1.—Frequency of published case reports on thrombotic thrombocytopenic purpura (55 cases to date)

TERMINOLOGY

The following designations have been proposed for the disease under discussion. diffuse, generalized, or disseminated platelet thromboses, by Baehr *et al* (8), Carter (18), and Gore (53), respectively, thrombocytic acroangiothrombosis, by Fitzgerald *et al* (43), thrombotic thrombocytopenic purpura, by Singer *et al.* (96), disseminated thrombocytic thrombosis, by Wyatt and Lee (126), disseminated platelet-cell thrombosis, by Comess and Oyamada (21), platelet thrombosis syndrome, by Beigelman (12), Moschcowitz syndrome, by Lennox and Dacie (66), thrombotic microangiopathic hemolytic anemia, by Symmers (109)

None of these terms is satisfactory. According to present knowledge, the vascular occlusions are not primarily composed of platelets and the lesions of the vascular wall are of great importance in the pathogenesis of the thrombi Any designation using the term "platelet thrombi" is therefore inaccurate The most commonly used term, "thrombotic thrombocytopenic purpura," does not emphasize the hemolytic anemia as an essential feature of the disease Meacham *et al* (70), criticizing the inadequacy of the last-mentioned designation, nevertheless point out that this name "has already acquired some

popularity, is short and alliterative A term describing all the components of the syndrome would be unwieldy, and the alternative of applying a proper name is generally objectionable." They propose "that the term thrombotic thrombocytopenic purpura be retained until a better understanding of the pathologic physiology of the condition permits the application of a more suitable name." With this suggestion we concur.

HISTOLOGIC PATTERN

The histologic picture consists of innumerable complete or, more frequently, incomplete occlusions of small arteries, arterioles, and capillaries by an amorphous or granular acidophilic material. Venules are rarely, if at all, involved (53, 76). The thrombi* may so dilate small arterioles that their walls become attenuated and the usual features distinguishing them from venous channels may disappear (53, 76) To avoid misidentification, contiguous sections of the vessel at a distance from the occluding and distending lesion should be studied, or its relation to other histologic structures, such as renal glomeruli or splenic follicles, be determined (53) Occasionally, the vascular wall may be ruptured, and the occluding material is then found in the adjacent connective tissue (18).

The dilatation of an affected vessel may take the form of a cylindrical, globular, or fusiform aneurysm, predominantly involving the arteriolo-capillary junctional zone (76) (Fig 2) The arteriolo-capillary junction appears to be a particularly vulnerable portion of the vascular tree in various hemorrhagic disorders. Humble (62) noticed that petechiae occurred with great regularity at this site in many thrombocytopenic as well as nonthrombocytopenic bleeding conditions. Thrombotic purpura seems to be no exception to this rule (53, 76).

The thrombotic lesions may be encountered in all organs of the body Most commonly they are seen in the myocardium, in the capsular zone of the adrenals, in the renal cortex, pancreas, and brain, where they are confined almost exclusively to the gray matter (3) They occur only rarely in the vessels of the lungs The hepatic sinusoids seem to be exempt, although the arterioles of the portal spaces may be affected (110)

* For convenience, the expressions "thrombus" and "vascular occlusion" are used synonymously, without regard to the chemical composition of the occluding mass The adjective "thrombotic" is employed as pertaining to vascular occlusion

Significantly, despite widespread dissemination of the vascular occlusions in an organ, the foci of ischemic parenchymal necrosis are predominantly microscopic. This lack of tissue degeneration has been attributed to the facts that the great majority of the vascular obstructions are incomplete and that the total capillary bed in a given area is only partially involved (14–18). No inflammatory reaction around the thrombosed vessels is noticeable. This absence of an exudative response is of diagnostic importance

The nature of the occluding material is still a subject of controversy.

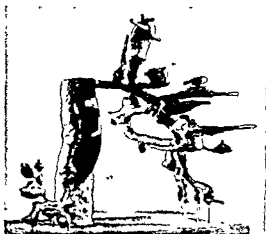


Fig 2.—Clay model representing a central artery with two of its branches. From each branch arise numerous capillaries distorted by aneurysms near their origin from the arterioles (76)

Moschcowitz (73), who first recognized the unique pattern of thrombotic purpura in 1925, described the thrombi as "hyaline" because they stain a uniform pink in hematoxylin-eosin preparations (Fig 3) He assumed them to be composed of agglutinated erythrocytes, an interpretation which was shared by Wiener as late as 1950 (121) Baehr, Klemperer, and Schifrin (8), in 1936, as well as almost all observers thereafter, studied the thrombotic material by means of various staining technics. With the phosphotungstic acid-hematoxylin stain the thrombi are pale brown, and with Weigert's stain, dull gray. Only occasionally is a bluish strand of fibrin detectable Mallory's aniline blue stain imparts a lavender color to the material and no fibrillar elements are noticeable Reticulum stains fail to reveal argentophil fibrils. With *Giemsa's stain the thrombi appear pale gray to bluish.*

The van Gieson technic gives a yellow hue to the occluding masses. Hemoglobin stains color the thrombi red or brownish red, in sharp contrast to the olive-green erythrocytes. Stains for amyloid exclude the presence of this protein With fat stains, no lipids are demonstrable in the thrombi The *Prussian blue reaction is negative, indicating* the absence of hemosiderin or related pigments On the basis of these tinctorial findings, the conclusion seems warranted that the thrombi are not composed of erythrocytes, leukocytes, fibrin, collagen, hemosiderin, or hemoglobin The hypothesis advanced by Baehr *et al.* (8)

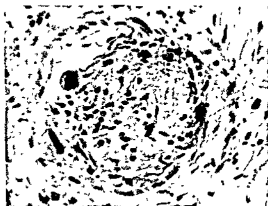


FIG 3 —Organized thrombus with megakaryocyte, myocardium, hematoxylin—eosin stain, ×300

was that the occlusions represent agglutinated platelets, and the term "diffuse platelet thromboses" was applied to this distinctive histologic pattern.

Gore (53) and Meacham *et al* (70) compared the staining reactions of the hyaline thrombi with those of a button of human platelets obtained by centrifuging platelet-rich plasma from which the red and white cells had been removed by differential centrifugation The platelet plug was fixed, embedded, and sectioned in the same way as the tissues The results obtained with the platelets were identical with those described above for the thrombi In addition, it could be shown that both the platelets and the occluding masses stain pink with the Schiff-periodic acid reagent, which reveals free aldehyde groups (53) Furthermore, with both materials, the Feulgen reaction is negative, signifying the absence of desoxyribose nucleic acid, and the fast

green stain, which identifies a basic substance, is positive (70). The toluidine blue stain shows polychromasia of the occlusions as well as of the platelets (70). Thus, the thrombocytes and the thrombi give identical staining reactions throughout and cannot be distinguished from each other by any of the numerous tinctorial technics so far used These histochemical findings, therefore, neither prove nor disprove that the thrombotic material is composed of agglutinated platelets (70, 76).

Of particular importance in the evaluation of the possible origin of the thrombotic masses are the changes in the walls of the affected vessels Associated with the occlusion, there are often found conspicuous proliferations of the vascular endothelium. Considering the thrombus formation to be the primary event, the proliferations have been assumed to encompass and later invade the thrombus (8, 114). The endothelial hyperplasia is, however, quite variable in degree. It may be entirely absent, or it may be slight to very marked. Some vessels show endothelial proliferation only, without any intraluminar thrombotic mass. At the margins of the thrombus, morphologically identifiable platelets can often be visualized. Carter (18) observed that some of the nonthrombosed vessels contained distinctly abnormal fibrin threads, which were three to five times thicker than normal fibrils and resembled hollow, nonsegmented mycelia That these bizarre masses were not mycelia could be established on morphologic and cultural grounds These findings have not been emphasized by other investigators Because of the frequent absence of any endothelial reaction, Baehr *et al.* (8), and later many other investigators, were disinclined to postulate the existence of a primary vascular lesion. With equal justification it may be reasoned that the finding of endothelial proliferations without thrombi suggests the presence of a primary vascular disorder (6, 70, 74, 76, 114).

Studies of serial sections demonstrate that the thrombus is often propagated free within the lumen for a considerable distance from its attachment to the vessel wall and may be covered by a sleeve of hyperplastic endothelial cells (53) (Fig 4) The presence of a focal abnormality of the endothelium from which the thrombus originates has been observed. Gore (53) described this "prethrombotic" lesion as a segmental accumulation of acidophilic material beneath the endothelium (Fig 5). This material may then begin to swell and protrude into the lumen, carrying with it the endothelial lining of the vessel. Further swelling causes a break in the endothelium which is then rapidly

covered with deposits of platelets. Thus a bimorphic thrombus originates, consisting partly of degenerated platelets and partly of the protruding substance formed in the vessel wall (53). The latter substance seems to be quite soluble in the blood stream, which accounts for the fact that most thrombi are not of the bimorphic but of the mono-

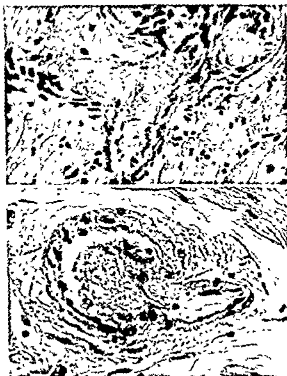


FIG 4 (above)—Thrombus extending the length of a vessel into its branches, myocardium, hematoxylin–eosin stain, ×300

FIG 5 (*below*)—Recent thrombus with "prethrombotic" lesion in vessel wall at point of branching, bimorphic thrombus, myocardium, hematoxylin–eosin stain, ×450.

morphic variety (53) The vascular lesion is interpreted as representing a pathologic alteration of the intercellular cement substance (53) Other studies indicate that at times the damage to the vascular wall may be even more intensive and that parts of the vascular structure, such as the elastic intima and even the media and adventitia, may become fragmented and be replaced by the acidophilic material (70, 76).

Judicial Co

Meacham *et al.* (70) consider the thrombi to be predominantly composed of extruded products of an "intermural degeneration rather than an intraluminal coagulum." Orbison (76), who first described the aneurysms in thrombotic purpura, emphasizes that the usual course of events in the development of any aneurysm begins with a destructive

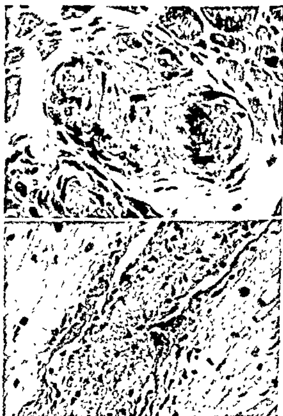


FIG. 6 (*above*) —Organized and recanalized thrombus, with superimposed recent thrombus, myocardium, hematoxylin–eosin stain, ×300

FIG. 7 (*below*) —Recent thrombus with changes in vessel wall at sites opposite the thrombus, myocardium, hematoxylin–eosin stain, ×300

lesion of the vascular wall, followed by dilatation and then by thrombosis. The observable loss of the elastic laminas in vessels with or without aneurysmal formation, or with or without thrombi, lends further support to the interpretation that the primary pathologic lesion lies in the wall of the vessel and that the thrombus formation is the

secondary event (76). To assume that the previously mentioned intramural changes are sequelae of platelet agglutination would require the postulation that thrombocytes could infiltrate and destroy the structures of blood vessels. Such a mechanism is not supported by any evidence, and is highly improbable (76).

It would be difficult to maintain the original "platelet thromboses" hypothesis in the light of this argument. The demonstration of the focal prethrombotic vascular lesion and of the formation of aneurysms has furnished new aspects to the histologic pattern of thrombotic purpura The concept that this disorder is characterized by a specific damage of the small caliber vessels seems well founded

Whatever the nature of the occlusions, most observers agree that the thrombi show various stages of aging and healing Recent thrombi are granular, the older occlusions are more compact, stain darker, and may look shrunken as if undergoing resorption (Figs. 6 and 7). Not infrequently, recanalization of such thrombi by endothelium lined spaces can be seen (Fig 6). The simultaneous presence of recent, organizing, organized, and recanalized occlusions gives the impression that the underlying process is repetitive and that the lesions occur in crops (6, 8, 53) This finding is of special interest in relation to the waxing and waning character of the hematologic, as well as of the neurologic, manifestations of the disease (95)

MORBID ANATOMY AND DIFFERENTIAL HISTOLOGIC DIAGNOSIS

At autopsy, petechiae and ecchymoses, the nonspecific signs of a hemorrhagic diathesis, are usually visible in many organs. Occasionally, there are subdural hematomas (54, 96) The valves of the heart may show friable, nonbacterial vegetations, which microscopically exhibit the specific histologic alterations (8, 9, 38, 45, 49, 54) The liver and spleen are frequently enlarged, the mesenteric and para-aortic lymph nodes infrequently Macroscopically identifiable, usually small foci of necrosis may be seen in the myocardium, brain, liver, spleen, pancreas, adrenals, and kidneys Sometimes the shape of the necrotic area is quite peculiar, involving, for example, only the outer third of the myocardium in a bandlike infarct (19) Acute nephritis has been noted (23, 53, 74, 116). In a number of instances, such unrelated diseases as tuberculosis (53, 112), sicklemia (53), pancreatitis (9, 21), acute bacterial endocarditis (17), old rheumatic heart disease (9),

Meacham *et al* (70) consider the thrombi to be predominantly composed of extruded products of an "intermural degeneration rather than an intraluminal coagulum" Orbison (76), who first described the aneurysms in thrombotic purpura, emphasizes that the usual course of events in the development of any aneurysm begins with a destructive

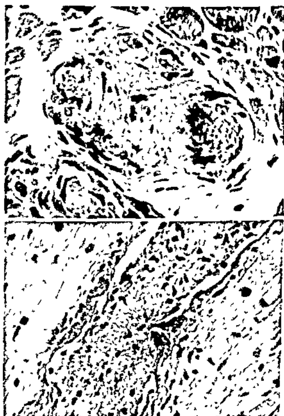


Fig 6 (*above*) —Organized and recanalized thrombus, with superimposed recent thrombus, myocardium, hematoxylin–eosin stain, ×300

Fig 7 (*below*) —Recent thrombus with changes in vessel wall at sites opposite the thrombus, myocardium, hematoxylin–eosin stain, ×300

lesion of the vascular wall, followed by dilatation and then by thrombosis. The observable loss of the elastic laminas in vessels with or without aneurysmal formation, or with or without thrombi, lends further support to the interpretation that the primary pathologic lesion lies in the wall of the vessel and that the thrombus formation is the

secondary event (76). To assume that the previously mentioned intramural changes are sequelae of platelet agglutination would require the postulation that thrombocytes could infiltrate and destroy the structures of blood vessels. Such a mechanism is not supported by any evidence, and is highly improbable (76).

It would be difficult to maintain the original "platelet thromboses" hypothesis in the light of this argument The demonstration of the focal prethrombotic vascular lesion and of the formation of aneurysms has furnished new aspects to the histologic pattern of thrombotic purpura The concept that this disorder is characterized by a specific damage of the small caliber vessels seems well founded.

Whatever the nature of the occlusions, most observers agree that the thrombi show various stages of aging and healing Recent thrombi are granular; the older occlusions are more compact, stain darker, and may look shrunken as if undergoing resorption (Figs 6 and 7) Not infrequently, recanalization of such thrombi by endothelium lined spaces can be seen (Fig 6) The simultaneous presence of recent, organizing, organized, and recanalized occlusions gives the impression that the underlying process is repetitive and that the lesions occur in crops (6, 8, 53). This finding is of special interest in relation to the waxing and waning character of the hematologic, as well as of the neurologic, manifestations of the disease (95)

MORBID ANATOMY AND DIFFERENTIAL HISTOLOGIC DIAGNOSIS

At autopsy, petechiae and ecchymoses, the nonspecific signs of a hemorrhagic diathesis, are usually visible in many organs Occasionally, there are subdural hematomas (54, 96) The valves of the heart may show friable, nonbacterial vegetations, which microscopically exhibit the specific histologic alterations (8, 9, 38, 45, 49, 54). The liver and spleen are frequently enlarged, the mesenteric and para-aortic lymph nodes infrequently. Macroscopically identifiable, usually small foci of necrosis may be seen in the myocardium, brain, liver, spleen, pancreas, adrenals, and kidneys. Sometimes the shape of the necrotic area is quite peculiar, involving, for example, only the outer third of the myocardium in a bandlike infarct (19). Acute nephritis has been noted (23, 53, 74, 110). In a number of instances, such unrelated diseases as tuberculosis (53, 112), sicklemia (53), pancreatitis (9, 21), acute bacterial endocarditis (17), old rheumatic heart disease (9),

Meacham *et al* (70) consider the thrombi to be predominantly composed of extruded products of an "intermural degeneration rather than an intraluminal coagulum" Orbison (76), who first described the aneurysms in thrombotic purpura, emphasizes that the usual course of events in the development of any aneurysm begins with a destructive



FIG 6 (*above*) —Organized and recanalized thrombus, with superimposed recent thrombus, myocardium, hematoxylin-eosin stain, ×300
FIG 7 (*below*) —Recent thrombus with changes in vessel wall at sites opposite the thrombus, myocardium; hematoxylin-eosin stain, ×300

lesion of the vascular wall, followed by dilatation and then by thrombosis The observable loss of the elastic laminas in vessels with or without aneurysmal formation, or with or without thrombi, lends further support to the interpretation that the primary pathologic lesion lies in the wall of the vessel and that the thrombus formation is the

of a family and generalized lupus (99) or polyarteritis (14) in another. The problem of the interrelationship between these diseases is certainly intriguing and deserves further close attention.

SYMPTOMATOLOGY

SEX, AGE, RACE.—As can be seen from Figure 1, reports on 55 cases in which autopsy revealed the histologic pattern of thrombotic purpura have been collected from the literature Contrary to earlier belief, the disease does not attack the female sex preferentially. of the 55 cases, 27 were in females and 28 in males. Nor is any race preference apparent, for it has been reported in both white and Negro individuals. Children, as well as adults, may be affected; age in the reported cases ranged from 9½ to 69 years.

ACUTE AND CHRONIC FORMS —It is now well established that thrombotic purpura may manifest itself clinically in two forms: (*1*) as an acute disorder which is fatal within a few days to weeks from onset, (*2*) as a more chronic process, of a relapsing nature, lasting for several months to years, then ending in death from an acute exacerbation. Whether there are milder episodes, from which complete recovery is possible, is not known

CLINICAL PICTURE OF ACUTE FORM

The signs and symptoms of acute thrombotic purpura can be conveniently divided into two groups of manifestations. The first group, which may be encountered in any thrombocytopenic purpura, comprises petechiae and ecchymoses of the skin and mucous membranes, retinal hemorrhages, and/or bleeding from body orifices. There may be epistaxis, hemoptysis, melena, vaginal or rectal hemorrhages, or hematuria All the laboratory evidence of the thrombocytopenic state, i.e., prolonged bleeding time, positive tourniquet test, and poor clot retraction, is usually present. However, some patients have thrombocytopenia, but only slight or even no purpura (15), and in a few exceptional instances neither diminution of the blood platelet level nor hemorrhagic manifestations were noticeable (49, 53). In the great majority of cases, however, thrombocytopenic purpura is the conspicuous clinical syndrome which brings the patient to the attention of the physician.

The features of the second group of symptoms and signs may make

nephrosclerosis (21), and malignant thymoma (23) have been found to co-exist with thrombotic purpura.

Vascular, nonexudative changes, resembling those of "platelet thrombosis," have been observed by Allen and Spitz (5) in scrub typhus (tsutsugamushi fever), confined, however, to the eschar, the primary lesion of this disease, and to the macular rash. Only rarely are such lesions also seen in the glomerular tuft capillaries of the kidneys, or in the minute septal vessels of the lungs (5). Extensive bacteriologic and serologic (complement fixation) studies, performed by Dr. Albert Milzer on one of my own patients with thrombotic purpura, yielded no indication of the presence of any rickettsial or viral infection (99).

The vascular lesions of thrombotic thrombocytopenic purpura can be readily differentiated from those of anaphylactoid (Schoenlein-Henoch) purpura, in which a cellular, inflammatory reaction is present around the affected vessels (46). Similarly, conspicuous cellular infiltrations in the vessel wall are seen in polyarteritis nodosa, where the nodularity may be caused by necrosis and granuloma formation as well as by aneurysmal dilatation. The infiltration by inflammatory cells, neutrophilic and eosinophilic granulocytes, and macrophages always affects the adventitia as well (7). This disease process is not restricted to the small caliber vessels. The thrombi found in polyarteritis and in Pagel's (78) "acronecrosis," a peripheral vascular disorder associated with subacute bacterial endocarditis, are composed predominantly of erythrocytes and fibrin, and thus show a tinctorial behavior quite different from that found in thrombotic purpura Pagel's fourth case should be considered as representing another instance of thrombotic purpura (86, 109). Hyaline thrombi may also be noticed in generalized lupus erythematosus, but the concentric, dense, fibrotic rings around the splenic arteries and the "wire loop" appearance of the renal glomeruli are the outstanding features of this collagen disease and are not encountered in typical instances of thrombotic purpura. However, probably because pathologists are now better acquainted with the histologic manifestations of this entity, a rapidly increasing number of observations describe the simultaneous existence of the vascular lesions of polyarteritis and/or lupus and of "disseminated platelet thrombosis" (9, 12, 19, 45, 50, 111, 116) On the basis of such findings, it has been suggested that the latter condition may also be assigned to the group of collagen diseases (12) In this regard, it is of particular interest that thrombotic thrombocytopenic purpura has been found in one sibling

dyspnea. Gallop rhythm has been recorded (12, 23, 38, 126). Systolic murmurs over the base of the heart are frequently heard. The possible existence of nonbacterial thrombotic endocarditis must be kept in mind. Chest pain due to myocardial infarction is uncommon, since the areas of necrosis are usually minute and only the small caliber vessels of the coronary system are affected. The electrocardiogram occasionally shows a prolonged P-R interval (38, 43); the T waves are sometimes of low voltage or inverted (9, 15, 38, 51), and the S-T segments elevated or depressed (9, 15, 51). In one instance, paroxysms of auricular tachycardia were demonstrable (49). The blood pressure is not altered by this disease.

5. *Respiratory system.*—Hemoptysis may be an early sign, but in general, this system is unmolested by the pathologic process.

6. *Gastrointestinal tract.*—Anorexia, nausea, diarrhea, and crampy abdominal pain constitute frequent complaints. Melena and bloody stools are manifestations of the purpuric condition. Glycosuria appeared in 1 patient with diffuse abdominal pain, and widespread necrosis of the islets of Langerhans was found at autopsy (36) Simultaneous occurrence of "pancreatitis" and thrombotic purpura has been reported (9, 21), but lesions of the pancreas extensive enough to give rise to clinical symptoms are exceedingly rare

7. *Genitourinary tract* —Vaginal bleeding may be the sole hemorrhagic symptom of any thrombocytopenic purpura, regardless of the etiology (27), and thrombotic purpura is no exception to this clinical rule (51). Microhematuria is almost invariably found, and gross hematuria is not infrequently observed. No instance of hemoglobinuria in this disorder has ever been reported, but hemoglobinemia may be present (4) The urinary sediment frequently contains an increased number of white cells and hyaline and granular casts. Nephritis has been encountered in association with thrombotic purpura (12, 18, 53, 74, 116), particularly in patients in whom postmortem examination disclosed the combination of changes indicative of generalized lupus or polyarteritis and of "platelet thrombosis" (12, 111, 116). In such instances, a so-called "telescoped" sediment (64) may be found in which all the elements ordinarily observed separately in different stages of nephritis are seen at the same time Renal insufficiency, resulting in moderate azotemia, is quite common, especially in the preterminal phase of the disease (9, 12, 15, 49, 70, 111, 112, 116).

8 *Mental and neurologic manifestations* —Almost all patients have some mental symptoms There may be restlessness, confusion, irrita-

the clinical diagnosis possible, and therefore require special consideration.

PRODROMATA.—Many patients give a rather vague history of an upper respiratory infection which precedes the appearance of the purpura. There may also be malaise, increased fatigability, generalized myalgia, arthralgia, throbbing headaches, and dizziness. Nausea, vomiting, and anorexia are often present. The temperature may be elevated to 100 to 102 F Urticaria (8, 43, 48, 49) and a localized, nonspecific ulcerative dermatitis (14, 49, 86) have been observed. In some patients the disease develops after parenteral administration of a foreign protein (smallpox vaccine (54), antitetanus serum (9), milk (112)); this probably is a fortuitous occurrence. Many have received sulfonamides or antibiotics before and during this prodromal period, hypersensitivity to sulfonamides and penicillin has been demonstrated in some (15, 38, 43).

PHYSICAL EXAMINATION AND RELATED LABORATORY STUDIES.—These may show the following abnormalities:

1. *Skin*.—There may be a few or numerous petechiae and ecchymoses. Baehr *et al* (8) have emphasized a peculiar, brownish café-au-lait color, but this is seldom noticeable.

2. *Pallor and jaundice*.—Pallor due to anemia is frequently a conspicuous feature In many patients the severity of the pallor is out of proportion to any observable loss of blood and sometimes precedes any hemorrhagic signs. Manifest or latent jaundice is also found quite regularly. The jaundice is always slight and of the acholuric, hemolytic type. Bilirubinuria is not demonstrable with the conventional methods, but the urobilinogen content of the urine may be increased

3. *Hepatosplenomegaly; lymphadenopathy*.—The liver and spleen were moderately enlarged in about half of the patients In the presence of hepatomegaly, the correct interpretation of the hemolytic jaundice, which is predominantly due to a hemolytic process, may be easily missed, particularly if the results of some liver function tests (bromsulfalein, cephalin flocculation, and thymol turbidity) indicate some concomitant liver damage (4, 23, 49, 70) The presence of an enlarged spleen practically excludes the diagnosis of acute idiopathic thrombocytopenic purpura, since in this disease splenomegaly is almost never encountered (59) Moderate enlargement of the lymph nodes accessible to palpation is occasionally observed (9, 14, 38, 43, 54, 86).

4 *Cardiovascular system*.—Acceleration of the pulse is noted in relation to the fever and the degree of anemia. There may be exertional

Hematologic Findings and Special Investigations

Hemoglobin and erythrocytes.—A moderate to severe anemia of the normochromic normocytic type is almost invariably present. The reticulocyte count is markedly elevated (up to 50 per cent), and many nucleated red cells may appear in the blood film. Exceptionally, the hemoglobin and red cell levels are normal but the reticulocyte output is high (48). The latter findings indicate a compensated hemolytic process in which the increased rate of erythrocyte production is still capable of balancing a not too markedly increased rate of red cell disintegration (24). There is conclusive evidence that the anemia is due to an intensive hemolytic mechanism of an unidentified nature. This is suggested by the following data:

1. *Changes in pigment metabolism.*—There is hemolytic jaundice which is combined with an elevated pigment excretion in the feces. The hemolytic index (72, 98), i.e.,

$$\frac{\text{Average fecal urobilinogen output/day}}{\text{total circulating hemoglobin}} \times 100,$$

which correlates the pigment excretion to its main source, is definitely much higher than normal (49, 70, 99).

2. *Intermittent spherocytosis.*—Spherocytic erythrocytes are readily seen in the blood films of some patients (4, 23, 48, 70, 74, 99, 116), but not in others with similarly severe anemia. Extensive daily studies of one of my cases (99), diagnosed ante mortem, demonstrated that spherocytosis may change in degree from day to day and may intermittently be entirely absent (Table 1). This phenomenon of intermittent spherocytosis does not occur to such an extent in any other spherocytic hemolytic anemia. However, its demonstration requires careful daily studies of the blood films. Since spherocytes can be interpreted as representing the prehemolytic state of erythrocytes (29, 92), their intermittent appearance points to a fluctuating intensity of the operating hemolytic mechanism during the course of the illness.

3. *Differential and mechanical fragilities of erythrocytes.*—Anomalies of the red cells often betray themselves in a differing pathologic resistance to various hemolytic agents, such as hypotonic saline solutions, lysolecithin, saponin, and acids. By means of these differential fragility tests (31, 93), characteristic reaction patterns have been obtained in some of the hemolytic syndromes. The differential fragility

bility, incoherent screaming, muttering delirium, stupor, or coma. These symptoms are usually transitory, and are followed by lucid intervals. Psychotic behavior may usher in the disease (9, 73), necessitating admission to a mental institution (51). Generalized (but not unilateral, or focal) convulsions are not infrequent. Besides these indications of a diffuse cerebral involvement, some patients show definite signs of focal lesions, such as vertigo, facial weakness, ptosis, hemiplegia, aphasia, apraxia, ataxia, dysphagia, or abnormal reflexes. Papilledema has been found occasionally (48, 99, 126). Significantly, however, even these organic signs are often transitory; they thus differ fundamentally from the cerebral manifestations (hemorrhage into the brain) seen in the other varieties of thrombocytopenic purpura, in which this waxing and waning of symptoms and signs is not observed Histologic studies of the brain suggest that the vascular occlusions take place in successive attacks, since recent as well as organizing and old thrombi are always demonstrable. Possibly, the intermittent character of the neurologic manifestations can be explained on this basis; by the opening of collateral channels, function may be restored with relative rapidity following such paroxysms of vascular obstructions (14, 99) Electroencephalography may reveal abnormal wave patterns, these have been interpreted as indicative of a diffuse cortical disorder (99), or even have sometimes aroused suspicion of a neoplasm (54). The cerebrospinal fluid is either normal or may show a positive Pandy reaction, slightly elevated protein content, and moderately increased cell count (3, 54). Xanthochromia is only rarely encountered (54). The values for glucose and chlorides always remain normal

9. *Fever*—Elevation of the temperature is consistently present throughout the course of thrombotic purpura It is usually moderate (100–102 F.), but terminal hyperpyrexia of 106 to 107 F may appear. In idiopathic thrombocytopenic purpura, prolonged fever is usually lacking or slight.

10 *Serum proteins.*—Essentially, no characteristic changes in the composition of the serum proteins have been encountered, except that occasionally the globulin values are high (17, 48).

11. *Bacteriologic and serologic features.*—Blood cultures have yielded no positive results in many instances. The Weil-Felix reaction, using Proteus OX[illegible] tive (9, 38, 99). [illegible] show false positive reactions (17, 23, 49).

red cells, these results reflect the previously mentioned phenomenon of intermittent spherocytosis. Obviously, single determinations of the osmotic fragility test may lead to erroneous conclusions. The transitory spherocytosis explains the conflicting statements in the literature about *the osmotic behavior pattern of the erythrocytes in thrombotic purpura.*

NATURE OF HEMOLYTIC MECHANISM.—Any hemolytic anemia is characterized by a shortening of the erythrocyte survival time (93). The time may be shortened because the patient's red cells are pri-

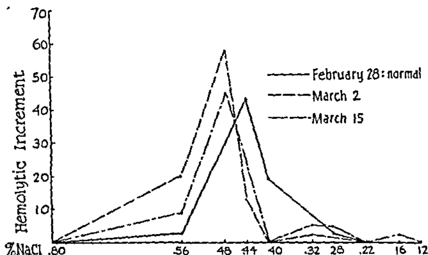


FIG 8.—Fluctuations of osmotic fragility of erythrocytes in thrombotic thrombocytopenic purpura (99).

marily defective (intracorpuscular mechanism) and therefore disintegrate more rapidly than normal ones (example: sickle cell anemia). The erythrocyte life span may also be shortened because of an extracorpuscular mechanism, by which an agent attacks and damages primarily normal erythrocytes (example: malaria). When normal red cells are transfused into a patient with a hemolytic syndrome due to an intrinsic defect of his own erythrocytes, the foreign cells survive normally. However, when compatible normal erythrocytes are transfused into individuals with an extracorpuscular hemolytic mechanism, the "foreign" cells are as rapidly and indiscriminately destroyed as the patient's own cells. All observers agree that, *in thrombotic purpura,* large blood transfusions are not effective in raising the red cell level

tests are based on the concept that pathologic erythrocytes, when injured by different procedures, react differently. Thus the lysolecithin fragility of "hereditary" spherocytes is greater than normal, whereas the resistance of spherocytes to this agent in acquired hemolytic disorders is not distinctive (55, 69, 92). The red cells of patients with paroxysmal nocturnal hemoglobinuria are very sensitive to a lowering of their environmental pH (56) so slight that it leaves other erythro-

TABLE 1.—INTERMITTENT SPHEROCYTOSIS IN PATIENT WITH THROMBOTIC THROMBOCYTOPENIC PURPURA

Date	Spherocytosis*	Nucleated Red Cells	Blasts	Promyelocytes	Myelocytes	Metamyelocytes
		per 100 White Cells				
Feb. 26	0	1	0	0	0	0
28	0	0	0	0	0	0
Mar 1	0	0	0	0	0	0
2	++	0	0	0	0	0
3	++	1	0	0	1	2
4	0	0	0	1	1	3
5	++	1	0	0	0	0
7	0	1	0	0	0	0
8	+	3	0	0	0	4
9	++	3	0	0	0	1
10	++	2	0	0	0	4
11	+++	0	0	0	0	0
12	+++	6	0	0	0	0
14	++++	8	0	0	2	2
15	++	13	0	0	4	7
16	+++	19	0	0	6	9
17	+	14	0	0	7	5
18	++	35	2	1	2	11

* Grading of spherocytosis + = 1 occasional spherocyte seen ++ = 1 to 2 spherocytes per oil immersion field +++ = several spherocytes per oil immersion field ++++ = numerous spherocytes

cytes entirely unaffected In many types of hemolytic disorders, regardless of their etiology, a decreased resistance of the red cells to trauma is demonstrable when they are rotated with glass beads under standardized conditions (mechanical fragility test (90)) The erythrocytes of patients with thrombotic purpura reveal an increased osmotic and mechanical fragility (4, 23, 48, 54, 74, 99), but a normal lysolecithin and acid fragility (98), this is the pattern of an acquired hemolytic process Interestingly, the osmotic fragility, although sometimes definitely increased, may show great variations from day to day (Fig. 8) Since the hypotonic fragility depends somewhat on the shape of the

tourniquet test, and decreased prothrombin consumption (4)—are almost always present in typical thrombotic purpura. Sometimes the tourniquet test is negative (49, 112) although the other abnormalities are present The specific histologic pattern has been observed without any apparent diminution of the thrombocytes (19, 49, 53, 111, 116). However, in some patients the thrombocytopenia has been found to be intermittent, the platelet count swinging between low and normal figures (12, 17, 38, 74, 109) As in other thrombocytopenic states, marked decrease of the platelet level may exist at times, with only scant or even without any hemorrhagic manifestations (48). Cases of thrombotic angiopathy without thrombocytopenia or purpura are quite unusual

Bedson's (11) animal experiments, confirmed by Elliott and Whipple (37), have demonstrated that lack of platelets, produced by intravenous injection of agar or antiplatelet serum, does not result in purpura. However, hemorrhages ensue if the capillary endothelium is simultaneously damaged by means of anti-red cell serum. Bedson (10) also found that antiplatelet serum in smaller doses produces thrombocytopenia only, whereas larger doses cause visible endothelial damage and hemorrhages as well Apparently therefore it is the vascular injury, leading to a wall, which is responsible for tl ental thrombocytopenic syndrome If it is permissible to apply these findings to clinical observations, diminution of platelet counts, with or without manifest purpura, may thus express a differing degree of the injurious effect of the same causative agent, thrombocytopenia without bleeding representing the milder damage.

However, this concept can be used only with difficulty to explain adequately the symptomatology of thrombotic thrombocytopenia in which there is little or no bleeding Although myriads of vascular occlusions are associated with a lack of platelets in these patients, hemorrhagic manifestations may nevertheless be absent or disproportionally slight. It may conceivably be postulated that the peculiar occlusions of the small caliber vessels have a sealing-off effect on the blood flow and that this may account for the discrepancy between vascular involvement, thrombocytopenia, and frank hemorrhage.

It is worth re-emphasizing here that the histologic pattern of thrombotic purpura is never encountered in any other type of purpura (8, 43, 75). Zucker (127) experimentally produced platelet thrombi in the cutaneous vessels of patients with idiopathic thrombocytopenic

considerably for any length of time, even in the absence of any gross bleeding. It therefore appears highly probable that an extracorpuscular hemolytic mechanism operates in this disorder (99).

Results of Coombs antiglobulin test.—This test demonstrates the presence of a globulin coat on the surface of the red cells (22, 95). It has been attributed in the spherocytic hemolytic anemias to the presence of autoimmune bodies directed against the erythrocytes (16, 22, 32, 95). These autoimmune bodies can be distinguished from the antibodies directed against the Rh-Hr system (95). Although it has been shown that a positive Coombs reaction may occur in hereditary spherocytosis (26, 95), such a finding is exceptional. In acquired hemolytic anemias of the idiopathic or symptomatic variety (94), a positive result of the antiglobulin test is rather commonly observed (32, 125), although sometimes very refined procedures (trypsinized cells) and acidification of the serum are necessary to demonstrate the globulin coating (32, 48). In thrombotic purpura, the Coombs test has been found to be negative in a number of patients (9, 15, 23, 49, 70, 99). Only Lennox and Dacie (66) and Gardner *et al* (48) have reported a positive result. These results may perhaps signify that the extracorpuscular hemolytic mechanism in this illness is not mediated by globulins (99), or that the damaging agent does not become fixed to the surface of the erythrocytes.

White cell changes.—The leukocyte count is normal or, more frequently, shows a moderate to marked elevation (up to 52,000 cells/cu mm (11)). A leukemoid reaction, characterized by the appearance of immature granulocytes (metamyelocytes, myelocytes, promyelocytes, and even myeloblasts) in the peripheral blood, has often been observed (8, 9, 14, 17, 38, 43, 49, 53, 54, 74, 96, 99, 114, 126). Daily blood studies (Table 1) reveal the intermittent appearance of this leukemoid reaction, the absence of such studies possibly explains why it has not been noticed quite regularly The finding of immature white cells in association with a hemolytic anemia and thrombocytopenia may suggest leukemia. The blood changes in acute leukemia are characterized by the presence of many blast cells, not by only a few immature granulocytes in all stages of myelopoiesis. The acute course of the illness militates against the diagnosis of chronic granulocytic leukemia

Platelet level and its relation to purpuric manifestations — The laboratory features of the thrombocytopenic syndrome—low platelet count, prolonged bleeding time, poor clot retraction, positive

caused by Sedormid the patient's serum harbors an antibody which will affect normal platelets *in vivo* and *in vitro,* but only in the presence of this drug Markedly decreased platelet counts are observed irregularly in acquired hemolytic anemias with globulin-coated erythrocytes Fisher (42) and Evans *et al.* (40, 41) suggested that the thrombocytopenia of such patients may be due to autoimmune bodies directed against their platelets Harrington *et al* (57) produced dramatic decreases of the thrombocyte levels, as well as purpuric manifestations, in normal recipients by transfusions with plasma from patients with idiopathic thrombocytopenic purpura However, not all plasmas of such patients elicited this effect These findings have been confirmed recently (101). Relatively simple *in vitro* technics have been devised for the demonstration of such plasma antiplatelet factors (58, 108)

In only 2 instances of proved thrombotic purpura have attempts been made to find platelet agglutinins in the patient's serum. Stats, in 1943, used cadaver blood of Bernheim's (14) patient, with negative results Adelson and Stefanini (4), employing the most modern technics, also could not detect an autoagglutinin against human platelets in their patient in antemortem studies.

HETEROPLATELET AGGLUTININ.—The last-mentioned investigators, however, discovered that although the serum of their patient with thrombotic purpura did not affect human platelets, it did agglutinate thrombocytes of dogs, rabbits, and rats. This heterophil antiplatelet antibody could be completely absorbed by guinea pig kidney, and incompletely by beef erythrocyte antigen In only one other instance of acquired hemolytic anemia with thrombocytopenia was a similar, but much less powerful, heterologous platelet antibody demonstrable. Serums from many patients with idiopathic or symptomatic thrombocytopenia did not contain such an antibody, but serums from individuals afflicted with infectious mononucleosis did The heterologous platelet antibody was associated with an increased titer of the antibodies against sheep erythrocytes Subsequently, the same authors pointed out that an abnormally high titer of the unabsorbed antisheep erythrocyte antibodies is by no means rare in various thrombocytopenic states (102) The possible diagnostic significance of the presence of a heterologous platelet agglutinin in thrombotic purpura requires further investigations

BONE MARROW FINDINGS —The marrow, obtained by the usual as-

purpura, but observed no similarity with the occlusions of the thrombotic disease. Consequently, the vascular lesions of the latter differ fundamentally from the vascular changes of other thrombocytopenic states

NATURE OF THROMBOCYTOPENIC MECHANISM.—Physiologically, the platelet level of the blood is kept relatively constant. This constancy is due to a balance between the rate of formation of thrombocytes by the megakaryocytes and their release into the circulation, and the rate of platelet disintegration. Diminution of the platelet count is therefore due to: (*1*) decreased production or release (44), or (*2*) increased destruction (63, 123), or (*3*) a combination of both mechanisms (40, 41, 58) In thrombocytopenia, the spleen has been accused either of inhibiting the manufacture of platelets (26, 67) or of removing them faster than normally from the circulation (33, 63, 123), or of both (58).

The physiologic life span of platelets in the circulation is probably 5 to 7 days, as has been demonstrated by transfusions of platelets into individuals whose thrombocyte level is in a steady state (60, 61, 65, 107). Eight different platelet agglutinogens have been distinguished on the basis of isoimmune bodies directed against these platelet agglutinogens (58, 104). The platelet agglutinogen system appears to be independent of the various red cell agglutinogen systems (ABO, CDEcde, etc.). In some thrombocytopenias, platelet transfusions reveal a normal survival time of the "foreign" thrombocytes, but in others they are very rapidly eliminated (58, 100, 105, 107) In the latter instances, thrombocytolysis may be assumed to exist, provided no platelet isoagglutinins are detectable in the recipient's plasma

In 1 patient with thrombotic purpura, diagnosed *in vivo*, Adelson and Stefanini (4) demonstrated an abnormally shortened survival (3 hr) of transfused normal platelets Although much more data of this kind are necessary for any definite conclusion, this finding suggests the existence in this disorder of an extrinsic platelet destroying mechanism

PLATELET AUTOAGGLUTININS —It is well established that in some, but by no means all, of the thrombocytopenic syndromes the patient's plasma contains platelet autoagglutinins which may differ in kind and appear to be responsible for the rapid disappearance rate of the thrombocytes (58, 108) Ackroyd (1, 2) demonstrated that in purpura

Demonstration of histologic vascular pattern in marrow specimens.—Attempts to find the vascular thrombi in the blood vessels of aspirated marrow particles were unsuccessful in one of my patients (99). Recently, however, Cooper *et al.* (23) visualized the characteristic pattern in paraffin sections of small fragments of material obtained by sternal marrow puncture. This procedure should be tried in all cases in which thrombotic purpura is seriously considered in the differential diagnosis (23, 99). Other locations for obtaining biopsy specimens, such as skin, gums, or muscles, seem less promising. Liver or spleen punctures are definitely contraindicated

Clotting factors.—The coagulation time is within normal limits in most patients with thrombotic purpura In 2 instances, a slight prolongation of the clotting time was noted (9, 49). Convincing proof for the presence of a circulating anticoagulant has not been reported A decrease of the prothrombin concentration to about 50 per cent of normal has been observed inconstantly (9, 23, 43, 70), usually in individuals with other evidence of liver damage. As in all sufficiently severe thrombocytopenic disorders, the prothrombin consumption is markedly decreased (4). No abnormalities of the plasma fibrinogen content have been noted (99). These results do not suggest any anomaly of the clotting mechanisms in thrombotic purpura.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF ACUTE THROMBOTIC PURPURA

The disease is by no means as uncommon as is generally assumed Although only 55 cases, verified by autopsy, form the basis of this report, the author has seen or heard of 8 more typical instances in the Chicago area within the last 2 years.

Recognition of the acute variety of thrombotic purpura during life is not too difficult, provided the symptomatologic triad, thrombocytopenic purpura, hemolytic anemia, and transitory bizarre neurologic signs, is fully developed The correct diagnosis has been established in a steadily increasing number of cases (4, 9, 15, 23, 38, 48, 66, 86, 99, 109).

The hemolytic character of the anemia is often fairly inconspicuous, particularly if the patient has lost considerable blood. However, prehepatic jaundice (hyperbilirubinemia without bilirubinuria) is an almost constant finding and should arouse suspicion of a possible

piration technic, shows a nonspecific pattern Since a hemolytic anemia exists, an increased percentage of nucleated red cell precursors of the normoblastic variety is regularly found Large amounts of hemosiderin may be visualized (4, 99). The granulocytic series may show a slight shift to the left. There are conflicting statements about the changes in the megakaryocytes. Some observers (4, 9, 36, 49, 70) record alterations like those encountered in idiopathic thrombocytopenic purpura (26, 79): an increased number of giant cells with a trend toward immaturity (a higher percentage of megakaryoblasts and promegakaryocytes), lack of granularity of these cells, and failure of platelet formation. Other investigators (6, 8, 43, 53, 54, 99) were not particularly impressed by such histologic anomalies. In some instances, megakaryocytes have been found in the lungs (14, 96, 114), in the heart (*see* Fig 3), and in the kidneys (126); but that has also been reported in other varieties of thrombocytopenias, as well as in many other diseases.

Inability of the megakaryocytes to form platelets has been attributed quite generally to splenic inhibition, and it has been postulated that a humoral factor exists which checks the manufacture and release of thrombocytes. However, a thrombocytolytic agent may not only remove platelets from the circulation but also from the marrow and, in addition, may cause some damage to the megakaryocytes (58). Tocantins and Stewart (113) noted marked degenerative changes in megakaryocytes during experimental thrombocytopenic purpura in dogs. Thus, diminished platelet production may be associated with a platelet-destroying mechanism

The conflicting findings of various competent investigators with regard to the activity of the megakaryocytes in thrombotic purpura could conceivably represent graded expressions of the effect of some agent damaging the thrombocytes as well as the megakaryocytes. The evidence for such a mechanism, however, is too inconclusive at present. The possibility that the giant cells change intermittently, similar to the intermittent spherocytosis described above, must also be considered These questions remain to be settled by future studies of the disease diagnosed in patients during life.

L E. CELLS —Neither in marrow preparations nor in those from peripheral blood have L E. cells ever been demonstrated, even when autopsy showed changes suggestive of the coexistence of disseminated lupus erythematosus and thrombotic purpura (9, 19, 23, 49)

TABLE 2.—AID TO DIFFERENTIAL DIAGNOSIS OF DISEASES WITH SIMULTANEOUS MANIFESTATIONS OF THROMBOCYTOPENIA AND HEMOLYTIC ANEMIA

DISORDER	DIAGNOSTIC FEATURES AND PROCEDURES					
	Spherocytosis	Osmotic Fragility	Coombs Antiglobulin test	Severity of Thrombocytopenia	Neurologic Signs	Other Features and Procedures
Thrombotic thrombocytopenic *purpura*	Intermittent	Intermittently increased	Usually negative	Usually severe	Transitory	Biopsy to establish histologic pattern
Idiopathic acquired hemolytic anemia	Present or absent	Increased or normal	Often positive	Usually slight, may be severe	Absent	—
Symptomatic hemolytic anemia Hodgkin's disease, leukemia, malignancy	Present or absent	Increased or normal	Usually positive	May be severe	May be present due to underlying disease, not transitory	Lymph node or splenic biopsy, demonstration of leukemic marrow, demonstration of tumor
Symptomatic hemolytic anemia disseminated lupus erythematosus	May be present	May be increased	May be positive	May be severe	Mental changes frequent	L E cells, characteristic skin lesions, polyserositis, telescoped urinary sediment
Symptomatic hemolytic anemia periarteritis nodosa	May be present	May be increased	May be positive	May be severe	Peripheral neuritis more frequent than cerebral involvement, not transitory	High eosinophilia in some instances, muscle biopsy
Drugs, e g , sulfanilamide	May be present	May be increased	Negative	May be severe	Absent	History of drug intake, Heins bodies may be found in red cells, hemoglobinuria may be present
Hereditary spherocytosis	Always present	Increased	Usually negative	Thrombocytopenia in crisis	Absent	Hereditary history
Paroxysmal nocturnal hemoglobinuria	Absent	Normal	Negative	Usually not severe	May be present due to thrombosis of cerebral vessels, not transitory	Positive acid fragility test, hemoglobinuria
Cirrhosis of liver	Absent	Normal	Negative	May be severe	Delirium may be present	Regurgitation jaundice, liver biopsy
Untreated pernicious anemia	Absent	Normal	Negative	Only in untreated cases slight to severe	Subacute combined disease, not transitory	Megalocytic marrow pattern

hemolytic syndrome, especially in the absence of any liver cell damage. Erythroid hyperplasia of the marrow and a rather high blood reticulocyte level, signifying increased production and release of erythrocytes, are readily demonstrable, but may be also encountered following a sufficiently severe hemorrhage. An elevated hemolytic index, intermittent spherocytosis and increased osmotic fragility, and the lack of response to blood transfusions will lead to the correct classification of the anemia.

The simultaneous occurrence of hemolytic anemia and thrombocytopenia is found in a number of other disease entities, and in some of them involvement of the nervous system may also be noticeable. Table 2 lists the pertinent clinical and laboratory features of the disorders which may be considered in the differential diagnosis. The waxing and waning type of neurologic manifestations is encountered exclusively in thrombotic purpura. Some of these entities, e.g., pernicious anemia, cirrhosis of the liver, and paroxysmal nocturnal hemoglobinuria, are ruled out quite readily. Studies of the survival time of the megalocyte (pernicious anemia erythrocyte) have established this anemia as a hemolytic syndrome (97). Thrombocytopenia is seen only in untreated, severe cases, and responds promptly to liver extract or vitamin B_{12}, the rise in platelets even precedes the elevation of the reticulocyte count in the peripheral blood. In cirrhosis of the liver, hemolytic anemia and thrombocytopenia may sometimes dominate the clinical picture (13, 94, 117, 120). Even in the presence of accelerated red cell disintegration, the icterus is usually of the regurgitation type, as manifested by bilirubinuria. Furthermore, in both cirrhosis of the liver and pernicious anemia, macrocytic hyperchromic red cells are usually present. The megaloblastic marrow pattern is quite diagnostic for pernicious anemia. In paroxysmal nocturnal hemoglobinuria, the free hemoglobin in the urine and the acid fragility test (56) are invaluable aids for differentiation. Diminution of the thrombocyte level is only rarely severe (84). Interestingly, neurologic signs may be produced by thromboses of larger cerebral vessels (88, 124). The mechanism responsible for these thromboses is not understood.

In hereditary spherocytosis, severe thrombocytopenia may be present during the aplastic crises (25, 77) which occur in this disorder. Usually the family history, the constant, marked spherocytosis, and the typical results of differential fragility tests permit the correct evaluation.

TABLE 2.—AID TO DIFFERENTIAL DIAGNOSIS OF DISEASES WITH SIMULTANEOUS MANIFESTATIONS OF THROMBOCYTOPENIA AND HEMOLYTIC ANEMIA

Disorder	Diagnostic Features and Procedures					
	Spherocytosis	Osmotic Fragility	Coombs Antiglobulin test	Severity of Thrombocytopenia	Neurologic Signs	Other Features and Procedures
Thrombotic thrombocytopenic purpura	Intermittent	Intermittently increased	Usually negative	Usually severe	Transitory	Biopsy to establish histologic pattern
Idiopathic acquired hemolytic anemia	Present or absent	Increased or normal	Often positive	Usually slight, may be severe	Absent	—
Symptomatic hemolytic anemia Hodgkin's disease leukemia malignancy	Present or absent	Increased or normal	Usually positive	May be severe	May be present due to underlying disease, not transitory	Lymph node or spleen biopsy, demonstration of leukemic marrow, demonstration of tumor
Symptomatic hemolytic anemia disseminated lupus erythematosus	May be present	May be increased	May be positive	May be severe	Mental changes frequent	L.E. cells, characteristic skin lesions, polyserositis, telescoped urinary sediment
Symptomatic hemolytic anemia periarteritis nodosa	May be present	May be increased	May be positive	May be severe	Peripheral neuritis more frequent than cerebral involvement, not transitory	High eosinophilia in some instances, muscle biopsy
Drugs, e.g., sulfanilamide	May be present	May be increased	Negative	May be severe	Absent	History of drug intake, Heinz bodies may be found in red cells, hemoglobinuria may be present
Hereditary spherocytosis	Always present	Increased	Usually negative	Thrombocytopenia in crisis	Absent	Hereditary history
Paroxysmal nocturnal hemoglobinuria	Absent	Normal	Negative	Usually not severe	May be present due to thrombosis of cerebral vessels, not transitory	Positive acid fragility test, hemoglobinuria
Cirrhosis of liver	Absent	Normal	Negative	May be severe	Delirium may be present	Regurgitation jaundice, liver biopsy
Untreated pernicious anemia	Absent	Normal	Negative	Only in untreated cases slight to severe	Subacute combined disease, not transitory	Megalocytic marrow pattern

hemolytic syndrome, especially in the absence of any liver cell damage. Erythroid hyperplasia of the marrow and a rather high blood reticulocyte level, signifying increased production and release of erythrocytes, are readily demonstrable, but may be also encountered following a sufficiently severe hemorrhage An elevated hemolytic index, intermittent spherocytosis and increased osmotic fragility, and the lack of response to blood transfusions will lead to the correct classification of the anemia.

The simultaneous occurrence of hemolytic anemia and thrombocytopenia is found in a number of other disease entities, and in some of them involvement of the nervous system may also be noticeable Table 2 lists the pertinent clinical and laboratory features of the disorders which may be considered in the differential diagnosis. The waxing and waning type of neurologic manifestations is encountered exclusively in thrombotic purpura. Some of these entities, e.g., pernicious anemia, cirrhosis of the liver, and paroxysmal nocturnal hemoglobinuria, are ruled out quite readily. Studies of the survival time of the megalocyte (pernicious anemia erythrocyte) have established this anemia as a hemolytic syndrome (97). Thrombocytopenia is seen only in untreated, severe cases, and responds promptly to liver extract or vitamin B_{12}, the rise in platelets even precedes the elevation of the reticulocyte count in the peripheral blood In cirrhosis of the liver, hemolytic anemia and thrombocytopenia may sometimes dominate the clinical picture (13, 94, 117, 120) Even in the presence of accelerated red cell disintegration, the icterus is usually of the regurgitation type, as manifested by bilirubinuria Furthermore, in both cirrhosis of the liver and pernicious anemia, macrocytic hyperchromic red cells are usually present The megaloblastic marrow pattern is quite diagnostic for pernicious anemia In paroxysmal nocturnal hemoglobinuria, the free hemoglobin in the urine and the acid fragility test (56) are invaluable aids for differentiation Diminution of the thrombocyte level is only rarely severe (84) Interestingly, neurologic signs may be produced by thromboses of larger cerebral vessels (88, 124). The mechanism responsible for these thromboses is not understood.

In hereditary spherocytosis, severe thrombocytopenia may be present during the aplastic crises (25, 77) which occur in this disorder Usually the family history, the constant, marked spherocytosis, and the typical results of differential fragility tests permit the correct evaluation

This patient died after a 5-year illness which showed remissions and exacerbations, and manifested itself with arthritis, fever, abdominal pain, mesenteric thrombosis, epilepsy, splenomegaly, and generalized lymphadenopathy. At times, the patient was anemic but not jaundiced, and thrombocytopenia was noted occasionally. Although at autopsy the specific pattern of thrombotic purpura was reported to exist, an acute bacterial endocarditis was also found. It is impossible to determine at what stage of this long-lasting ailment thrombotic purpura really developed. I have found only 2 well-documented cases in the literature (48, 70) which showed remissions of long duration (2½ years and 9 months, respectively), both following splenectomy. When the symptoms recurred, careful restudy of the histologic sections of the splenic tissue established the diagnosis of thrombotic purpura Subsequent autopsies revealed the features characteristic of this disorder.

In the first of the 2 cases, a patient of Meacham *et al.* (70) was admitted to the hospital with fever, hepatosplenomegaly, and severe hemolytic anemia (Hb, 3 8 Gm; RBC, 1,080,000; reticulocytes, 14 5 per cent; increased osmotic fragility of the erythrocytes). The platelets appeared diminished in the film, but the bleeding time was normal and neither purpuric manifestations nor neurologic abnormalities were observed. Transfusion of 7 L of blood did not appreciably change the red cell level Splenectomy was then performed The course following the operation was stormy, but eventually the red cell level began to rise During the next 2 years the patient was asymptomatic, with an erythrocyte count fluctuating between 3,000,000 and 4,000,000 Relapse occurred, with development of mental and neurologic symptoms and signs. There was no anemia, but the reticulocyte count was high, suggesting a compensated hemolytic process. The platelet level was about 50,000 per cubic millimeter. The direct Coombs test was negative There were *periods of slight improvement followed by new* exacerbations, and death came 3 months after the second hospital admission.

The second, even more remarkable case, a patient of Gardner *et al.* (48) was that of a 24 year old woman who was hospitalized at the age of 8 for acquired hemolytic anemia (RBC, 1,800,000, reticulocytes, 28 per cent, osmotic fragility of the red cells markedly increased) Spontaneous recovery occurred within a few weeks. At the age of 19, the patient was again jaundiced, but for 2 weeks only At that time she noticed that urticaria developed when she ate tomatoes

The idiopathic and symptomatic types of acquired hemolytic anemias, and sometimes drug reactions (e.g., sulfanilamide) may provide really great difficulties in differential diagnosis. Thrombocytopenic purpura is by no means as uncommon in these conditions as was formerly believed. In drug purpura with hemolytic anemia, demonstration of Ehrlich-Heinz bodies within the erythrocytes may sometimes be helpful (52, 118). Symptomatic hemolytic anemia due to leukemia, Hodgkin's disease, or neoplasm may resemble quite closely the disease under discussion (94). The leukemoid reaction seen in thrombotic purpura can be easily misinterpreted. The Coombs antiglobulin test is usually positive in symptomatic hemolytic disorders, and negative in thrombotic disease, but this laboratory result is not particularly valuable since there are many exceptions to the rule. Transient neurologic manifestations are not encountered in the nonthrombotic syndrome The correct diagnosis is often made from biopsy specimens which reveal the neoplastic tissue.

The greatest difficulties arise in differentiating thrombotic purpura from instances of disseminated lupus erythematosus and polyarteritis nodosa with symptomatic hemolytic anemia and thrombocytopenic purpura (28, 34, 35, 71, 85, 89, 119). The pathogenic mechanisms operating in these three diseases are probably quite similar, since they attack the red cells, the platelets, and the blood vessels. That the angiopathy of thrombotic purpura may sometimes appear together with the vascular changes of generalized lupus or polyarteritis has already been noted. L E. cells have neither been reported in thrombotic purpura nor in polyarteritis. Marked eosinophilia suggests the latter. Polyserositis or the characteristic skin lesions may help in the diagnosis of generalized lupus. It is in these diagnostic situations, however, that demonstration of the characteristic histologic pattern of a noninflammatory vascular occlusion in a marrow biopsy specimen may be the only means to arrive at the correct *in vivo* evaluation of the symptomatology of thrombotic purpura

CHRONIC INTERMITTENT THROMBOTIC PURPURA

The course of most reported cases of thrombotic purpura was usually a rapid one, with death occurring in a few days to less than 8 weeks. A few patients had a more prolonged illness, up to about 100 days, with short intervals of subjective and objective improvement (38, 110). The case of Brown and Norman (17) is difficult to classify

TREATMENT

EFFECT OF SPLENECTOMY.—This operation is an empirically found procedure which is sometimes of great therapeutic value in the hemolytic anemias and thrombocytopenic purpuras. Whereas in hereditary spherocytosis removal of the spleen is always successful in stopping the rapid disintegration of the defectively built erythrocytes, its effect in acquired hemolytic states is unpredictable on purely clinical grounds, varying from a complete cure to no particular influence on the disease process (93, 95). Most observers now agree that a similar situation exists for the different thrombocytopenic syndromes.

When it was demonstrated that in some acquired hemolytic anemias the patient's plasma contains factors directed against his own red cells (autoagglutinins or hemolysins), the spleen could be shown to be occasionally the sole or main site of production of these factors (115). In such instances, the Coombs antiglobulin test, positive prior to the operation, becomes negative afterward and the anemia disappears (48, 68, 95, 115), in other, less fortunate cases, the manufacture of the antired cell factors is not confined predominantly to the spleen, and removal of this organ influences the rate of red cell destruction only negligibly or not at all (95). However, there are patients with acquired hemolytic anemia whose red cell level cannot be raised by extensive blood transfusions, but whose serum does not seem to contain any antired cell factors readily demonstrable with the technics presently available Frequently, the hemolytic anemia in thrombotic purpura appears to belong to this latter category

Similar situations prevail in some thrombocytopenic syndromes The spleen may produce autoantiplatelet factors, splenectomy may then be lifesaving by removing the sole or main production center of these damaging agents (58) However, the spleen may not be the only organ concerned in manufacturing these factors, and then splenectomy may be of no, or doubtful, value Evidence is also available that the spleen removes sensitized platelets (58) and probably also red cells Harrington *et al* (57, 58) demonstrated that transfusions of human plasma containing a powerful antiplatelet agent caused severe and sustained thrombocytopenia in both normal and splenectomized recipients. Transfusion of a less potent thrombocytopenic plasma induced diminution of the platelets in nonsplenectomized recipients only (58). Similar results were obtained in animals with the use of antiplatelet serums (58). Splenectomy, however, is also sometimes

or took aspirin A febrile episode of severe spherocytic anemia developed 5 years later (Hb, 3.1 Gm.; platelets, 72,000 per cu. mm) It was possible to demonstrate a hemolysin in the patient's acidified serum which was active against normal as well as her own erythrocytes. The Coombs test was strongly positive Facial weakness and hemiparesis appeared. Then the unusual feature of severe thrombophlebitis of both arms and legs, with multiple pulmonary infarcts, became manifest. The patient improved somewhat under ACTH therapy, but bilateral papilledema and retinal hemorrhages began to complicate the picture. Because of the noticeable hematologic relapse, splenectomy was performed. The effect of the operation was dramatic: the Coombs test became negative and the anemia disappeared. The platelet level did not rise remarkably. There was a symptom-free interval of 9 months Then progressive disorientation and severe purpura brought the patient again to the hospital. There was no anemia. Death occurred after 4 days. Autopsy and restudy of the splenic sections showed the typical pattern of thrombotic purpura (47).

This case history is unique as it describes 2 or possibly 3 attacks of acquired hemolytic anemia with symptom-free intervals of several years duration, the last attack was definitely due to the thrombotic disease. This is also the second instance of a positive Coombs test in thrombotic purpura The dramatic effect of splenectomy on the hemolytic anemia is quite similar to the result observed in the patient of Meacham *et al.* (70). Even when the neurologic and purpuric manifestations recurred, the anemia was only slight or absent following the operation These findings document again the independence of the hemolytic mechanism and the processes responsible for the thrombocytopenia and the vascular occlusions Perusal of these reports also gives rise to the suspicion that quite a number of patients who died from acquired hemolytic anemia in the past might have shown the histologic features of thrombotic angiopathy, which, however, were not recognized at that time From these experiences, the clinical rule may be derived that the more chronic variety of the thrombotic disorder should be considered in all patients who show intermittent attacks of hemolytic anemia and/or purpura Paraffin sections from aspirated marrow particles may establish the diagnosis on an objective basis. Regular performance of this technic, now in little use, may help to solve the problem whether milder cases of thrombotic disease, with possibly lasting recovery, exist.

this treatment have been poor. Nevertheless, further trials with these hormones, using large dosages (starting with 300 to 500 mg of cortisone in adults), are definitely indicated

BLOOD AND PLATELET TRANSFUSIONS.—Blood transfusions may have to be given to sustain life, but usually cannot elevate the red cell level for any long period of time on account of the extracorpuscular hemolytic mechanism Platelet transfusions (60, 103), although not generally available, are probably of no value since the platelets are removed very rapidly from the circulation in thrombotic purpura (4)

ANTICOAGULANTS.—Since vascular occlusions are a main feature of the disease, the idea of trying anticoagulants preventively appears intriguing Heparin has been recommended (99) and has been used without effect (4). It may even tend to produce more bleeding because of the existing thrombocytopenic purpura Since most probably the occlusions are caused by a primary pathologic reaction of the vascular wall to some damaging agent, and since the thrombi are not composed of fibrin, it is extremely doubtful whether anticoagulants could prevent such a process

SULFONAMIDES AND ANTIBIOTICS.—Because the course is febrile, most patients receive antibacterial treatment The sulfonamides, penicillin, aureomycin, and streptomycin have not been effective in thrombotic purpura (9, 21, 38, 43, 49, 86, 99, 109, 110, 116) and may even be detrimental when there is hypersensitivity to these drugs (36, 38, 43, 49). It should be kept in mind that some antibiotics (aureomycin, terramycin) suppress the transformation of bilirubin to stercobilinogen in the gut by interfering with the intestinal flora (87) This side effect may render evaluation of laboratory studies of the pigment metabolism quite difficult (95), and, if not taken into consideration, may give rise to erroneous interpretations.

PATHOGENESIS AND ETIOLOGY

Little is known about the causative mechanisms operating in thrombotic purpura Any hypothesis dealing with its pathogenesis must explain (1) the combined appearance of vascular occlusions, thrombocytopenic purpura, and hemolytic anemia, and (2) the fluctuations in the intensity of the pathologic processes which are reflected in the conspicuous waxing and waning character of the clinical manifestations

The original "platelet thromboses" hypothesis (8) assumed that the occlusions were composed exclusively of platelets and that the throm-

effective in patients without any demonstrable thrombocytolytic agents (58). In such cases, the existence of splenic inhibition factors regulating platelet production and release has been postulated as an explanation.

Against this general background of our present knowledge of the possible role of the spleen in acquired hemolytic and thrombocytopenic disorders, the effect of splenectomy in proved instances of thrombotic hemolytic thrombocytopenic purpura must be evaluated. This operation has been performed in 9 patients (8, 9, 38, 48, 54, 70, 74, 112), in 7, no effect was noticeable and death occurred in the operating room or a few hours or days afterward. In 2 cases (48, 70) splenectomy appears to have been beneficial in inducing temporary remissions. However, purpura was not an outstanding feature in these two individuals at the time of surgery. Anemia was absent when relapse occurred, and the purpura and neurologic involvement dominated the clinical picture.

It is obvious that more data are necessary before an opinion can be formed about the advisability of splenectomy in thrombotic purpura. Practically, it is probably best to remove the spleen as early as possible after the diagnosis has been made. The surgeon and the patient's relatives should be told that death may follow rapidly after operation, but that there is a slight chance of inducing a temporary improvement.

ACTH AND CORTISONE.—It is now well established that these hormones are highly beneficial in many cases of acquired hemolytic anemias (26, 48, 83, 122). Disappearance of the serum antired cell factors and of the anemia have been observed Cessation of hormone administration may or may not lead to relapse. In thrombocytopenic states the hormones frequently tend to decrease capillary permeability (82, 106, 122) The bleeding time may return to normal, although the platelet level often continues to remain low. Disappearance or diminution of antiplatelet factors has also been observed (58) If splenectomy is considered in a hemolytic and/or thrombocytopenic syndrome, it may be advantageous to start hormone treatment for a few days preoperatively and then perform the operation, continuing hormone administration for some time in the postoperative period, depending on the response of the patient

The effects of ACTH and cortisone in thrombotic purpura are difficult to evaluate (4, 9, 23, 48, 49, 70) Some transitory improvements have been reported (48), but it must be kept in mind that the natural course of this disease is episodic. In most instances, the results with

their dissimilarities. Hemolytic anemia and thrombocytopenic purpura are relatively uncommon in patients with lupus or polyarteritis, whereas in thrombotic purpura the specific vascular lesions are only exceptionally not associated with the characteristic hematologic abnormalities. This is more suggestive of some qualitative rather than quantitative difference in the causative agents. The pathogenic situation may be somewhat comparable to that seen in hemolytic disease of the newborn. If the mother becomes sensitized due to some component of the Rh-Hr system, the erythrocytes of the infant become coated with the respective antibodies. If the ABO system is involved, the damaged red cells of the infant will give a negative Coombs reaction (39), although the hemolytic process is basically caused by the same pathophysiologic mechanism. This example has also been chosen to illustrate the fact that the negative Coombs reaction observed in the hemolytic anemia of thrombotic purpura does not preclude the existence of a powerful extracorpuscular hemolytic mechanism of an unidentified nature. Similarly, the absence of demonstrable antiplatelet autoagglutinins does not rule out the possible presence of a potent plasma thrombocytolysin. It would be tempting to transfuse plasma of patients with thrombotic purpura into normal volunteers to see whether the disease can be reproduced, analogous to the approach of Harrington *et al.* (57) in their study of the pathogenesis of idiopathic thrombocytopenic purpura.

Our knowledge of the disease is too incomplete to explain satisfactorily its episodic course. Rich (81) has pointed out that "in the case of lesions in which hypersensitivity constitutes the actual pathogenic mechanism of injury, the etiologic agent may be quite different in different cases." However, great caution must be used in implicating drugs as causative factors, even though they may have been shown to cause hypersensitivity reactions in experimental animals. The fact that thrombotic purpura developed shortly after a patient used an iodine-containing compound does not necessarily prove a causal relationship (36) The history, obtainable in some patients, of hypersensitivity reactions to the sulfonamides or penicillin, of a tendency to urticaria, or of coexisting glomerulitis (74) neither proves nor disproves that thrombotic purpura belongs to the group of hypersensitivity diseases The significance of the vague upper respiratory infection or of the use of foreign protein which sometimes precedes the development of the symptoms of the disease is not understood. Since the *in vivo* diagnosis of thrombotic purpura is now made more often, it may be hoped

bocytes were consumed in the formation of the thrombi. No explanation was offered for the agglutination of the platelets. The anemia was interpreted as being due to loss of erythrocytes into the tissues, and destruction of these extravasated red cells was held responsible for the acholuric icterus. It was pointed out that similar vascular occlusions are encountered in experimental animals in which the Shwartzman phenomenon (91) had been induced. However, in these sensitized animals, hemorrhage and leukocytic infiltrations of the vessel walls precede the formation of thrombi, which are located in the venules. In the disease in man, the arterioles and capillaries are predominantly involved. Furthermore, it is now established that the occlusions are not merely due to agglutinated platelets, since the vascular wall is definitely and, most probably, primarily affected, and that the anemia is caused by an extracorpuscular hemolytic mechanism which also damages transfused normal red cells at a rapid rate. Clinical and histologic observations further suggest that there is no parallelism between the damage to the blood vessels, the extent of thrombus formation, and the degree of thrombocytopenia, purpura, and anemia. The rare instances of thrombotic angiopathy without lack of platelets in the circulation, and/or with no or mild anemia, indicate that the disease process is unlikely to be due to a single causative factor.

Other diseases involving the blood vessels are associated irregularly with hemolytic anemia and thrombocytopenic purpura Some patients with disseminated lupus erythematosus or with polyarteritis nodosa may show a clinical picture very similar to that of thrombotic purpura (28, 34, 35, 71, 85, 89). According to Rich's (81) fundamental concept, polyarteritis and lupus may be considered manifestations of hypersensitivity mechanisms Rich and associates have been able to produce necrotizing vascular lesions in experimental animals by means of a variety of agents, such as foreign proteins, sulfonamides and iodine. It therefore seems conceivable that a hyperergic reaction may also be the fundamental mechanism of thrombotic purpura. The simultaneous appearance of histologic changes considered to be characteristic of lupus and polyarteritis, and of thrombotic purpura, is noteworthy, as is the occasional tendency of members of one family to become afflicted with any one of these diseases.

Beigelman (12) believes that "platelet thromboses," polyarteritis, [illegible] same fun- [illegible] imilarity of [illegible] n stressing these disorders. [illegible]

19 Case records of the Massachusetts General Hospital Case 38301, New England J. Med. 247:133, 1952.
20 Claugh, P W.: Thrombotic thrombocytopenic purpura. Ann. Int. Med 33 739, 1950 (editorial).
21 Comess, O H, and Oyamada, A Uremia and disseminated platelet-cell (thrombocyte) thrombosis, A.M.A. Arch. Int Med 89 802, 1952
22 Coombs, R R A, Mourant, A E and Race, R R. A new test for detection of weak and "incomplete" Rh agglutinins, Brit. J Exper. Path. 23.255, 1945.
23 Cooper, T., et al Thrombotic thrombocytopenic purpura, Am J Med 13 374, 1952
24 Crosby, W H, and Ackroyd, J. F. The limits of hemoglobin synthesis in hereditary hemolytic anemia, Am. J Med., 13.273, 1952
25 Dameshek, W, and Bloom, M L The events in the hemolytic crisis of hereditary spherocytosis with particular reference to the reticulocytopenia, pancytopenia, and abnormal splenic mechanism, Blood 3 1381, 1948
26 Dameshek, W, and Miller, E. B The megakaryocytes in idiopathic thrombocytopenic purpura, a form of hypersplenism, Blood 1 27, 1946
27 Dameshek, W, and Rheingold, J. J. Idiopathic thrombocytopenic purpura and menorrhagia mistakenly treated for local disease, J.A.M.A. 139 993, 1949
28 Dameshek, W, and Rosenthal, M C Treatment of acquired hemolytic anemia With a note on the relationship of periarteritis nodosa to hemolytic anemia M Clin North America 35.1423, 1951
29 Dameshek, W., and Schwartz, S O: Hemolysins as the cause for clinical and experimental hemolytic anemia Am J M Sc 196 769, 1938
30 Dameshek, W, Rosenthal, M C, and Schwartz, L. L. The use of ACTH in the treatment of acquired hemolytic anemia with abnormal antibodies, New England J Med 244 117, 1951
31 Dameshek, W, Schwartz, S O, and Singer, K Spherocytosis (and increased erythrocyte fragility) as indicators of hemolytic activity, with consideration of "differential fragility," J Clin Invest. 18.479, 1939
32 Davidsohn, I, and Oyamada, A Specificity of auto-antibodies in hemolytic anemia, Am J Clin Path 23 101, 1953
33 Doan, C A, Curtis, G M, and Wiseman, B K The hemolytopoietic equilibrium and emergency splenectomy, J A M A 105 1567 1935
34 Dubois E L Systematic lupus erythematosus, M Clin North America 36 1111, 1952
35 Dubois, E L Acquired hemolytic anemia as the presenting syndrome of lupus erythematosus disseminatus, Am J Med 12 197, 1952
36 Ehrich, W E, and Seifter, J Thrombotic thrombopenic purpura caused by iodine, Arch Path 47 446 1949
37 Elliott, R H E, and Whipple, M A Observations on interrelationship of capillary, platelet, and splenic factors in thrombocytopenic purpura, J. Lab. & Clin Med 26 489, 1940
38 Engel, G. L, Scheinker, I M, and Humphrey, D C Acute febrile anemia and thrombocytopenic purpura with vasothromboses, Ann Int Med 26 919, 1947
39 Erythroblastosis fetalis [round table discussion], Pediatrics 10 337, 1952

that further experimental studies into the etiology and pathogenesis of this interesting disorder may be forthcoming.

REFERENCES

1 Ackroyd, J F. The pathogenesis of thrombocytopenic purpura due to hypersensitivity to Sedormid, Clin Sc. 7 249, 1949
2. Ackroyd, J. F Allergic purpura, including purpura due to foods, drugs and infections, Am J Med 14 605, 1953.
3. Adams, R D, Cammermeyer, J., and Fitzgerald, P. J The neuropathological aspects of thrombocytic acroangiothrombosis, J. Neurol, Neurosurg & Psychiat 11 27, 1948.
4. Adelson, E, and Stefanini, M Studies on platelets: VI Demonstration and characterization of a heterologous (Forssman) platelet agglutinin, Blood 7 700, 1952
5 Allen, A, and Spitz, S A comparative study of the pathology of scrub typhus (tsutsugamushi disease) and other rickettsial diseases, Am J Path 21 603, 1945
6 Altschule, M D A rare type of acute thrombocytopenic purpura Wide-spread formation of platelet thrombi in capillaries, New England J Med 227 477, 1942
7 Anderson, W A D *Textbook of Pathology* (St Louis C V Mosby Company, 1948)
8 Baehr, G, Klemperer, P, and Schifrin, A An acute febrile anemia and thrombocytopenic purpura with diffuse platelet thromboses of capillaries and arterioles, Tr A Am Physicians 51 43, 1936
9 Barondess, J A Thrombotic thrombocytopenic purpura, Am J Med 13 - 294, 1952
10 Bedson, S P Effect of splenectomy on production of experimental purpura, Lancet 2 1117, 1924
11 Bedson, S P Blood-platelet anti-serum Its specificity and role in the experimental production of purpura, J Path & Bact 25 94, 1922
12 Beigelman, P M Variants of the platelet thrombosis syndrome and their relationship to disseminated lupus, A M A Arch Path 51 213, 1951
13 Berman, L, *et al* The blood and bone marrow in patients with cirrhosis of the liver, Blood 4 511, 1949
14 Bernheim, A I Wide-spread capillary and arteriolar platelet thrombi, J Mt Sinai Hosp 10 287, 1943
15 Blackman, N S, Cohen, B M, and Watson, J Thrombotic thrombopenic purpura, J A M A 148 546, 1952
16 Boorman, K E, Dodd, B E, and Loutit, J F Hemolytic icterus (acholuric jaundice), congenital and acquired, Lancet 1 812, 1946
17 Brown, E B, and Norman, J W Multiple platelet thrombi, New York J Med 46 2167, 1946
18 Carter, J R Generalized capillary and arteriolar platelet thrombosis, Am J M Sc 213 585, 1947

acute "self-limited" and chronic types, A M A Arch Int. Med 88.701, 1951
60 Hirsch, E O, and Gardner, F H.. The life span of transfused human platelets, J. Clin Invest 30 649, 1951
61. Hirsch, E. O; Favre-Gilly, J, and Dameshek, W. Thrombopathic thrombocytopenia, successful transfusions of blood platelets, Blood 5 568, 1950
62. Humble, J. G · The mechanism of petechial hemorrhage formation, Blood 4.69, 1949
63 Kaznelson, P Verschwinden der haemorrhagischen Diathese bei einem Fall von *"essentieller Thrombopenie" (Frank) nach Milzextirpation. Splenogene* thrombolytische Purpura, Wien. Klin Wchnschr 29 1451, 1916
64 Krupp, M. A Urinary sediment in visceral angitis (periarteritis nodosa, lupus erythematosus, Libman-Sacks "disease") Quantitative studies, Arch Int Med 71·54, 1943
65 Lawrence, J, and Valentine, W N The blood platelets The rate of their utilization in the cat, Blood 2 40, 1947
66 Lennox, B, and Dacie, J V.. Case presented at Internat Cong of Clinical Pathology, London, July, 1951.
67. Limarzi, L R, and Schleicher, E M The reaction of peripheral blood and bone marrow in chronic hemorrhage and in essential thrombopenic purpura, J A M A 114 12, 1940
68 Loutit, J F, and Mollison, P L Haemolytic icterus (acholuric jaundice), congenital and acquired, J Path & Bact 58 711, 1946
69. Maier, C · Der Lysolecithintest bei hamolytischen Anamien, Helvet med acta 14 470, 1947
70 Meacham, G C, *et al* Thrombotic thrombocytopenic purpura, Blood 6 706, 1951
71. Michael, S R, *et al* The hematologic aspects of disseminated (systemic) lupus erythematosus, Blood 6 1059, 1951
72 Miller, E B, Singer, K, and Dameshek, W Use of the daily fecal output of urobilinogen and the hemolytic index in the measurement of hemolysis, Arch Int Med 70 722, 1942
73 Moschcowitz, E An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries, Arch Int Med 36 89, 1925
74 Muirhead, E E, Crass, G, and Hill, J M Diffuse platelet thromboses with thrombocytopenia and hemolytic anemia (thrombotic thrombocytopenic purpura), Am J Clin Path 18 523, 1948
75 Nickerson, D A, and Sunderland, D. A The histopathology of idiopathic thrombocytopenic purpura hemorrhagica, Am J Path 13 463, 1937
76 Orbison, J L Morphology of thrombotic thrombocytopenic purpura with demonstration of aneurysms, Am J Path 28 129, 1952
77 Owren, P. A. Congenital hemolytic jaundice The pathogenesis of "hemolytic crisis," Blood 3 231, 1948
78 Pagel, W Acronecrosis due to fibrin thrombi and endothelial cell thrombi, Am J M Sc 218 425, 1949
79 Presley, S J, Best, W R, and Limarzi, L R Bone marrow in idiopathic thrombocytopenic purpura, J. Lab & Clin Med 40 503, 1952

40. Evans, R S., and Duane, R. T.: Acquired hemolytic anemia: I. The relation of erythrocyte antibody production to activity of the disease, II The significance of thrombocytopenia and leukopenia, Blood 4·1196, 1949.
41. Evans, R. S, *et al*. Primary thrombocytopenic purpura and acquired hemolytic anemia, evidence for a common etiology, A M.A. Arch Int. Med 87:48, 1951.
42. Fisher, J. A. Cryptogenetic acquired hemolytic anemia, Quart J. Med 16 245, 1947.
43. Fitzgerald, P J; Auerbach, O, and Frame, E: Thrombocytic acroangiothrombosis (platelet thrombosis of the capillaries, arterioles, and venules), Blood 2·519, 1947
44. Frank, E.: Die essentielle Thrombopenie. (Konstitutionelle Purpura, Pseudo-Hamophilie), Berlin. Klin. Wchnschr. 52 454 and 490, 1915.
45 Friedberg, C K., and Gross, L: Nonbacterial thrombotic endocarditis associated with acute thrombocytopenic purpura, Arch. Int Med 58 641, 1936.
46 Gairdner, D: The Schoenlein-Henoch syndrome (anaphylactoid purpura), Quart. J. Med. 17 95, 1948.
47 Gardner, F. Personal communication
48 Gardner, F. H, *et al.* The effect of adrenocorticotrophic hormone (ACTH) in idiopathic acquired hemolytic anemia as related to the hemolytic mechanism, J. Lab. & Clin Med 37.444, 1951.
49 Gendel, B. R., Young, J M, and Kraus, A P.· Thrombotic thrombocytopenic purpura, Am J Med 13 3, 1952.
50 Gitlow, S, and Goldmark, C Generalized capillary and arteriolar thrombosis, report of two cases with a discussion of the literature, Ann. Int. Med 13 1046, 1939
51 Goldenberg, P T, Thayer, J E, and Hastings, L. P. Febrile thrombopenic purpura with hemolytic anemia and platelet thrombosis, New England J Med 243 252, 1950
52 Good, M G: Heinz body phenomenon in erythrocytes, Blood 5 885, 1950
53 Gore, I. Disseminated arteriolar and capillary platelet thrombosis, morphologic study of its histogenesis, Am J Path 26 155, 1950
54 Green, M A, and Rosenthal, S Generalized blood platelet thrombosis, J Mt Sinai Hosp 16 110, 1949
55 Gripwall, E: Zur Klinik und Pathologie des hereditaren hamolytischen Ikterus mit besonderer Berucksichtigung des Verhaltens der roten Blutkorperchen Acta med Scandinav supp 96, 1938.
56 Ham, T. T. Studies on destruction of red blood cells, Arch Int Med 64 1271, 1939.
57 Harrington, W. J, *et al* Demonstration of a thrombocytopenic factor in the blood of patients with idiopathic thrombocytopenic purpura, J Lab & Clin Med. 36.1, 1951.
58. Harrington, W J, *et al* Immunologic mechanisms in idiopathic and neonatal thrombocytopenic purpura, Ann Int Med 38 433, 1953
59 Hirsch, E O, and Dameshek, W "Idiopathic" thrombocytopenia Review of 89 cases with particular reference to the differentiation and treatment of

102 Stefanini, M, and Adelson, E · Studies on platelets VIII. Heterophile reaction due to Forssman antibody in patients with idiopathic and secondary thrombocytopenic purpura, Am J Clin. Path. 22·1164, 1952.
103 Stefanini, M, and Dameshek, W · Collection, preservation and transfusion of platelets, New England J. Med 248 797, 1953
104 Stefanini, M, and Plitman, G · Antigenicity of platelets and evidence for platelet groups in humans, J Clin. Invest. 32 606, 1953
105 Stefanini, M., Dameshek, W, and Adelson, E. Platelets, VII Shortened "platelet survival time" and development of platelet agglutinins following multiple platelet transfusions, Proc Soc Exper. Biol & Med 80 230, 1952
106 Stefanini, M, *et al* Corticotropin (ACTH) in idiopathic thrombocytopenic purpura, J A M A. 149 647, 1952
107. Stefanini, M, *et al*. Studies on platelets II The effect of transfusion of platelet-rich polycythemic blood on the platelets and hemostatic function in "idiopathic" and "secondary" thrombocytopenic purpura, Blood 7 53, 1952
108 Stefanini, M, *et al* · Studies on platelets IX Observations on the properties and mechanism of action of a potent platelet agglutinin detected in the serum of a patient with idiopathic thrombocytopenic purpura (with a note on the pathogenesis of the disease), Blood 8 26, 1953
109 Symmers, W S C Thrombotic microangiopathic hemolytic anemia (thrombotic microangiopathy), Brit M J 2 897, 1952
110 Symmers, W S C, and Barrowcliff, D F. Platelet thrombosis syndrome, J Path & Bact 63 552, 1951
111 Symmers, W S C, and Gillett, R. Polyarteritis nodosa, associated with malignant hypertension, disseminated platelet thrombosis "wire loop" glomeruli, pulmonary silicotuberculosis, and sarcoidosis-like lymphadenopathy, A M A Arch. Path 52 489, 1951
112 Tacket, H S, and Jones, R S Thrombocytic acroangiothrombosis, Circulation 5 920, 1952
113 Tocantins, L M, and Stewart, H L Pathological anatomy of experimental thrombopenic purpura in the dog, Am J Path 15 1, 1939
114 Trobaugh, F E, *et al* An acute febrile illness characterized by thrombopenic purpura, hemolytic anemia and generalized platelet thrombosis, Arch Path 41 327, 1946
115 Wagley, P F, *et al* Studies on the destruction of red blood cells VI The spleen as source of a substance causing agglutination of the red blood cells of certain patients with acquired hemolytic jaundice by an antihuman serum rabbit serum (Coombs serum), J Lab & Clin Med 33:1197, 1948
116 Wallace, D C Diffuse disseminated platelet thrombosis (thrombotic thrombocytopenic purpura), with a report of two cases, M J Australia 2 9, 1951
117 Watson, C J Hemolytic jaundice and macrocytic hemolytic anemia, certain observations in a series of 35 cases, Ann Int Med 12 1782, 1939
118 Webster, S H Heinz body phenomenon in erythrocytes, Blood 4.479, 1949
119. Wechsler, M S *A Textbook of Clinical Neurology* (6th ed, Philadelphia W B Saunders Company, 1947)
120 Whitesell, F B, and Snell, A M. Thrombopenia and increased capillary fragility in hepatic disease, J A.M A 140 1071, 1949

80 Rackow, F., Steingold, L, and Wood, J H. F.: Thrombotic thrombocytopenic purpura, Acta med Scandinav 143 137, 1952
81. Rich, A R. Hypersensitivity in diseases, Harvey Lect 42 106, 1947.
82 Robson, H N, and Duthie, J J. R. Capillary resistance and adrenocortical activity, Brit M J 2 971, 1950
83 Rosenthal, M C, *et al* The use of adrenocorticotropic hormone and cortisone in the treatment of leukemia and leukosarcoma, Blood 6 804, 1951
84 Ross, J F. Hemoglobinemia and hemoglobinurias, New England J Med 233 691, 1945
85 Ross, S W, and Wells, B B. Systemic lupus erythematosus, Am J Clin Path 23 139, 1953
86 Rotter, R Clinicopathologic conference, Wisconsin M J. 49 1035, 1950.
87. Sborow, V M, Jay, A. R, and Watson, C J. The effect of aureomycin on urobilinogen formation and the fecal flora, J. Lab & Clin Med 34 1743, 1949
88 Scott, R B, Robb-Smith, A H. T, and Scowen, E. F The Marchiafava-Micheli syndrome of nocturnal hemoglobinuria with hemolytic anemia, Quart J Med 7.95, 1938
89 Shearn, M H, and Pirofsky, B. Disseminated lupus erythematosus, A M A. Arch Int Med 90 790, 1952
90 Shen, S C, Castle, W B, and Fleming, E M Experimental and clinical observations on increased mechanical fragility of erythrocytes, Science 100 387, 1944
91 Shwartzman, G *Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological and Clinical Significance* (New York Paul B Hoeber, Inc, 1937), p 167
92 Singer, K The lysolecithin fragility test, Am J M Sc 199 466, 1940
93 Singer, K Problems of erythrocyte disintegration with particular reference to the life span of the red cell, J Lab & Clin Med 30 784, 1945
94 Singer, K, and Dameshek, W Symptomatic hemolytic anemia, Ann Int Med 15 544, 1941
95 Singer, K, and Motulsky, A G The developing (Coombs) test in spherocytic hemolytic anemias, J Lab & Clin Med 34 768, 1949
96 Singer, K, Bornstein, F P, and Wile, S A Thrombotic thrombocytopenic purpura Hemorrhagic diathesis with generalized platelet thromboses, Blood 2 542, 1947
97 Singer, K, King, J C, and Robin, S The life span of the megalocyte and the hemolytic syndrome of pernicious anemia, J Lab & Clin Med 33 1068, 1948
98 Singer, K, Miller, E B, and Dameshek, W Hematologic changes following splenectomy in man, Am J M Sc 202 171, 1941
99 Singer, K, Motulsky, A G, and Shanberge, J N Thrombotic thrombocytopenic purpura II Studies on the hemolytic syndrome in this disease, Blood 5 434, 1950
100 Sprague, C C, *et al* Platelet transfusion and the pathogenesis of idiopathic thrombocytopenic purpura, J A M A 150 1193, 1952
101 Stefanini, M.· Recent observations on the pathogenic mechanism in idiopathic thrombocytopenic purpura, J Mt Sinai Hosp 19 452, 1952

Porphyria

C. J. WATSON

Department of Medicine, University of Minnesota, Minneapolis

THE TERM porphyria embraces a group of diseases characterized by peculiar clinical features, together with excessive formation and excretion of uroporphyrin and coproporphyrin and/or certain chromogens or precursors. As emphasized by Garrod (51), porphyria is best regarded as an inborn error of metabolism, though it is difficult to determine whether porphyria is not at times an acquired disease rather than one depending on a constitutional abnormality.

Reference to the earlier literature relating to porphyria may be found in several recent reviews (23, 85, 142, 156, 158), and need not be considered in detail here. Hoppe-Seyler (66) first used the term "porphyrin" in purely descriptive fashion to designate the iron-free compound which makes its appearance when blood is treated with concentrated sulfuric acid. This was called hematoporphyrin. It was subsequently crystallized by Nencki and Sieber (89), and is a well-defined entity. Although earlier cases had been observed in which there was great excess of porphyrin in the urine, Gunther (60–62) was the first to study the disease in any detail and to call it "hematoporphyria." While Gunther (62) recognized that there were slight spectroscopic differences between what he called the "natural hematoporphyrin" found in these cases and the hematoporphyrin of Hoppe-Seyler and Nencki, it nevertheless became widely accepted that the substances were identical. Hans Fischer (31, 32) first crystallized the porphyrins from the excreta of a patient with porphyria and clearly demonstrated that they differed from hematoporphyrin. Fischer's observations were made in the now famous case of the patient named Petry, which was an example of Gunther's "porphyria congenita." In-

121. Wiener, A. S.: Auto-antibody formation and disease, Brit. M. J. 2:163, 1950 (letter to the editor).
122. Wintrobe, M. M, *et al.*. Effect of corticotrophin and cortisone on the blood in various disorders in man, Arch Int. Med 88 310, 1951.
123. Wiseman, B. K, Doan, C A, and Wilson, S J.. The present status of thrombocytopenic purpura with special reference to diagnosis and treatment, J A M A. 115 8, 1940.
124. Witts, L J: The paroxysmal hemoglobinurias, Lancet 2.115, 1936
125. Wright, C S, *et al* Studies of hemagglutinins in hereditary spherocytosis and in acquired hemolytic anemia, their relationship to the hypersplenic mechanism, J Lab & Clin Med 37 165, 1951.
126. Wyatt, J. P, and Lee, R. S.. Hemorrhagic encephalopathy due to disseminated thrombocytic thrombosis, Arch. Path 49 582, 1950.
127. Zucker, H D. Platelet thrombosis in human hemostasis, A hematologic study of skin wounds in normal and purpuric individuals, Blood 4 631, 1949

1937, Waldenstrom *et al* (140–142) isolated a substance from the urine of patients with acute porphyria which they regarded as type III uroporphyrin It is now clear, however, that this porphyrin is an association or molecular complex of porphyrins of types I and III. The whole question of the nature and significance of the Waldenstrom porphyrin has become a matter of controversy and will be considered in detail later.

Of significance in the problem of human porphyria is the nature of the porphyrin of turacin, the copper complex of the uroporphyrin found in the wing feathers of the touracos (*Turacus*). Turacin was first isolated by Church (16). Fischer and Hilger (36) removed the copper and reported that the porphyrin was uroporphyrin I, but still later Rimington stated that it was uroporphyrin III (92, 106). Rimington found only uroporphyrin III in turacin, and regards it as an "unequivocal" reference compound for the study of other uroporphyrins Over a number of years, various samples of turacin have been studied with Dr Schwartz in this laboratory The results of this study will be reported elsewhere, in brief, it has shown that the uroporphyrin from the turacin studied by Fischer and Hilger (36), some of which was available to us, was indeed uroporphyrin I, yielding only coproporphyrin I on decarboxylation. On the other hand, turacins from other species have in some instances yielded only uroporphyrin III, and in still others from 60 to 80 per cent uroporphyrin I, the remainder being the type III isomer The reason for this variation is unknown, and whether genetic or environmental must be determined by future study. Obviously this may have certain implications for the problem of porphyria in man

FORMS OF PORPHYRIA

Until recently, the various proposed classifications of porphyria have been largely clinical in character, as may be seen from Table 1, which lists the three most widely used classifications Actually, those of Gunther (61, 62) and of Waldenstrom (142) are the most generally accepted

Recent studies by Schmid and associates (115) in this laboratory have provided data of fundamental significance with regard to the tissue porphyrins in porphyria. When correlated with other available information about the various forms of the disease, this study is believed to provide a basic classification which comprises a primary

deed, Gunther (62) had already made a detailed clinical study of this patient. Fischer isolated two new porphyrins, one of which he found only in the urine (31) and the other mainly in the feces and in small quantity in the urine (32). He designated these uroporphyrin and coproporphyrin, respectively. Although it was evident that coproporphyrin was excreted in the urine as well as in the feces, many years elapsed before a uroporphyrin was demonstrated in the feces of patients with porphyria (120). Fischer and associates (35, 41, 43, 45) showed that the configuration of the substituent groups of the uro- and coproporphyrin isolated in the case Petry differed from that of the underlying protoporphyrin of the hemoglobin molecule. For structural relationships, the reader is referred to recent reviews (156, 158). Compared with the four artificial etioporphyrin isomers, which possess only methyl and ethyl groups in varying positions, the uroporphyrin and coproporphyrin of the case Petry corresponded with type I, while the hemoglobin protoporphyrin is of the type III series (45, 156). This was subsequently proved by synthesis, both of the four coproporphyrins and of the hemoglobin protoporphyrin (39, 45) Uroporphyrin I was readily decarboxylated to coproporphyrin I, and it became evident that there was a "dualism" of the porphyrins in nature (35, 41) Thus, in the case Petry it was clear that while the uro- and coproporphyrin of the excreta were of the type I series, the hemoglobin protoporphyrin was type III, nor was it possible to demonstrate any belonging to type I. Much the same situation was later shown to exist in yeast cells (38), especially when undergoing autolysis, coproporphyrin I being formed in considerable amount, but not at the expense of the type III hemin, which was also present. Twenty years later Kench and Wilkinson (69) made the important observation that yeast cells can be made to alter the preponderance of copro- I or III formed by changes in environment, more particularly by the relative amount of carbohydrate and potassium in the media This is a point of some importance in considering the dualism in animal species, such as the *Turacus*, or in animals with experimental porphyria, and possibly even in human porphyria

For a time it appeared that the free porphyrins of the excreta might be limited to the type I series Van den Bergh and co-workers, however, reported isolation of coproporphyrin III in cases of "congenital porphyria" (40, 134, 135), and a short time later Grotepass (59) isolated this porphyrin from the urine of patients with lead poisoning, a finding that has been widely confirmed (151) In the years 1935 to

porphyria purely on the basis of
was 40 years old at the time of
recently re-examined the report
photosensitive type. Of these, only about a fourth could be classified with a fair degree of certainty as erythropoietic In many, the features were much more indicative of the hepatic type, in some, the data were insufficient to permit classification. It has been generally held that porphyria congenita is much more common in males, but again, because of the confusion with the hepatic cutanea tarda variety, there is little doubt that restudy of the sex incidence in this disease is needed. For purposes of comparison, 160 cases of all types on which we have had opportunity to collect data (71 were studied in Minneapolis, the remainder on the basis of protocols and urine samples received from colleagues elsewhere) have been divided according to

TABLE 2 —SEX INCIDENCE AND TYPE OF DISEASE IN 160 CASES OF PORPHYRIA

TYPE	NO OF CASES	MALE	FEMALE
Porphyria erythropoietica	5	1	4
Porphyria hepatica	155	77	78
Intermittent acute	97	38	59
Cutanea tarda	23	19	4
Combined or mixed	11	6	5
Latent	23	14	9

sex (Table 2). It is seen that of the 5 cases of the erythropoietic type, 4 were in females. Actually, the term "congenital," which has generally been applied to these cases, is unsatisfactory. In a good number, the symptoms fail to appear for several years after birth, and though it is quite likely that the trait is present at birth, this is at least as true of the hepatic types.

Quot. Photosensitivity is prominent in this form of the disease, often appearing very early in life, at times within a few days after birth, but often not until after an interval of a year or two In a number of instances, the urine apparently did not become dark or red until after some exposure to light While this relationship has never been established beyond question, it has been strongly suggested in certain cases, for example, Anderson's (4) case, the first to be reported in which hydroa and porphyrinuria were definitely associated, and in a case studied in this laboratory (115) This point is of interest in relation to the effect of light in enhancing experimental porphyrinuria

The early lesions of photodynamic origin are the blisters of hydroa

division into two main groups, porphyria erythropoietica and porphyria hepatica. The latter is then further subdivided, partly on a clinical basis and partly on a biochemical one. The studies have been carried out in the main on bone marrow and liver biopsy material. Information about porphyrin content has been gained by direct fluorescence microscopy and by microanalytic methods of assay, involving extraction, fractionation, and fluorimetry.

Studies of typical cases of Günther's "porphyria congenita," such as the case Petry, have clearly revealed that the abnormal porphyrin formation occurs in the developing red cells of the bone marrow, while in all other forms, including the acute, the chronic or cutanea tarda,

TABLE 1.—PREVIOUS CLASSIFICATIONS OF PORPHYRIA

GÜNTHER (62)	WALDENSTRÖM (142)	MICHELI-DOMINICI (87)
Acute	Acute, with variations	Idiopathic
Toxic	Cutanea tarda	Abdominal
Idiopathic	Congenital	Nervous
Chronic		Cutaneous
Congenital		Toxic

and the combined or mixed type, the abnormal formation of porphyrin and porphobilinogen is believed to take place in the liver.

In the light of this newer information, the following classification seems permissible·

- Porphyria erythropoietica
- Porphyria hepatica
  - Intermittent acute
  - Cutanea tarda
  - Mixed or combined type
  - Latent

PORPHYRIA ERYTHROPOIETICA

This form is very rare It is exemplified by the case Petry, the best known instance of Gunther's "porphyria congenita." The prominence of photosensitivity in this type of porphyria has caused it to be confused with the hepatic photosensitive or cutanea tarda type of the disease, so that the latter, although fundamentally distinct, has often been mistaken for the erythropoietic type Many examples of this might be cited. One that is especially pertinent is the case of van den Bergh *et al.* (134, 135), in which coproporphyrin III was found for the first time. This was designated as an instance of "congenital"

type I isomer. (The problem of porphyrin biosynthesis in this and other forms of porphyria is discussed later.) The bone marrow was hyperplastic-normoblastic. After splenectomy, the excessive hemolysis disappeared, the bone marrow returned to normal, and the porphyrin formation declined to a very small fraction of that before operation. With this, the photosensitivity disappeared. This patient has remained entirely well for 4 years, although subsequent examinations of the urine and feces have revealed small but significant amounts of uroporphyrin, together with a mild increase of coproporphyrin in the feces. Prior to splenectomy, the red blood cells and the patient's

to operation. Nevertheless, there has been cessation of excessive hemolytic activity and reduction of photosensitivity for 2 years. The beneficial effect on photosensitivity in these cases was rather unexpected, but is believed to be of fundamental significance. It is reasonable to suppose that it relates to a marked reduction in porphyrin formation; this, in turn, is based on reduced erythropoiesis, a result of the splenectomy which eliminated increased hemolytic activity. In both of our cases, the spleens were found to contain large amounts of uro- and smaller amounts of coproporphyrin. The concentrations were approximately the same as those observed in the bone marrow. The porphyrin concentration in the liver, however, was considerably smaller. Typical data are shown in Table 3. The amounts in the spleen could not be related to extramedullary erythropoiesis, as this was minimal in both patients. They were perhaps best explained, however, as due to the destruction of porphyrin-laden erythrocytes and consequent porphyrin storage. The smaller amounts in the liver were also regarded as blood derived (115).

The porphyrin formation in the bone marrow normoblasts was most convincing (115). Chemical assay on the myeloid-erythroid layer revealed an extremely high concentration of uro- and coproporphyrin; *in the fluorescence microscope, red fluorescence of varying intensity* was observed in the cytoplasm of many of the normoblasts and to an even greater extent in the nuclei (Fig. 1). Some normoblasts, however, *failed to reveal any fluorescence.* Dr. Schmid recognized that the fluorescing normoblasts in these cases revealed curious nuclear abnormalities (116, 118) which thus far have only been seen in this disease. These consist of small, well-defined vacuoles or peculiar faults

estivale (hydroa vacciniforme) on skin surfaces exposed to light, especially of the face and hands (158). In the earlier literature, the disease was likened to pemphigus or leprosy. In time, scarring and mutilation increase, partly as a result of secondary infection. After years of continued photosensitivity, the mutilation becomes extensive, with loss of fingers, portions of the nose, ears, scarring of the cheeks and about the mouth, ectropion, or symblepharon Illustrations of the lesions have been published repeatedly (14, 62, 88, 156) In the late stage, the appearance is not unlike that of advanced xeroderma pigmentosum Skin not exposed to light remains unaffected. While this aspect of the disease is spectacular from the standpoint of mutilation, it is seldom fatal, death usually being due to intercurrent disease or to anemia Increased hemolytic activity or outspoken hemolytic anemia is frequently observed. Indeed, hemolytic anemia and splenomegaly probably constitute an integral feature of the disease, for they appear sooner or later in most cases. Mackey and Garrod (83) emphasized the progressive enlargement of the spleen. In the famous case Petry, the anemia was regarded as "pernicious" by Borst and Konigsdorffer (8). These authors first noted the porphyrin-laden nucleated red cells in the bone marrow, which they believed to be megaloblasts. The illustrations, however, are more suggestive of normoblasts or erythroblasts, and the evidence that this was pernicious anemia rather than hemolytic anemia seems inadequate Splenomegaly and bone marrow hyperplasia (erythropoietic) were outspoken In retrospect, the demonstration of the erythropoietic origin of the porphyria in the case Petry, seems relatively clear, though the extensive deposits of porphyrin throughout his body tended to mask the relationship, at least as compared with what has been found in much earlier stages, such as studied in this laboratory (2, 115)

In some cases, as in one studied recently by Schmid et al (115), there is clear evidence of increased hemolytic activity and erythropoiesis without anemia, in other words, a "compensated" hemolytic state The important role of the spleen in relation to the hemolytic anemia and photosensitivity, at least in some cases of the erythropoietic type, has become apparent only within recent years Splenectomy was performed in 2 of our cases, 2 and 4 years ago (2, 115) In the first case, a severe hemolytic anemia threatened life, in addition, there was marked photosensitivity with early cutaneous scarring. The urinary and fecal content of uroporphyrin and coproporphyrin were exceedingly high, and consisted of about 99 per cent

suggests that it is a purely physical phenomenon. Mackey and Garrod (83) observed that red fluorescence may be seen in the phalangeal bones if a strong source of ultraviolet light is allowed to shine through the fingers. This method has not been sufficiently employed. There is reason to believe that it would also yield positive results in cases of



FIG. 1.—Erythropoietic porphyria. *Above*, unstained preparation of bone marrow, photographed in the fluorescence microscope under ultraviolet light, the white areas represent red fluorescence (116) *Below*, Wright-stained bone marrow preparation, central normoblast showing hemoglobin inclusion

the hepatic cutanea tarda type. Thus Paul *et al.* (96a) have recently described red bones in such an instance studied at autopsy.

Erythrodontia may be observed in those cases in which sufficient porphyrin has been deposited in the teeth to make them grossly red, or reddish brown. Mackey and Garrod regarded this as evidence that excessive porphyrin formation was already in progress in the fetus, but this does not seem an altogether safe conclusion Teeth which

in the nuclei (Fig. 1). Dr. Schmid has now observed these abnormalities not only in the 2 cases studied in this laboratory but in material from the cases of Zuelzer and Kaplan (164) in Detroit, and of Gray and Neuberger (55) in London, sent to him through the kind cooperation of these investigators. The nature and absolute or relative specificity of these changes remain to be determined.

The porphyrin concentrations in the bone marrow, as shown in Table 3, were so large, compared with those in the circulating erythrocytes and plasma, that the liberation of porphyrin by intact normoblasts during maturation seems probable, when their relation to the daily output of porphyrin is considered. Any other assumption would require a fantastic destruction of normoblasts and/or erythrocytes. Direct examination of the bone marrow, however, failed to yield evidence that normoblasts were disintegrating, and the apparent rate of destruction of circulating erythrocytes was quite inadequate to account for the magnitude of porphyrin excretion.

TABLE 3.—TISSUE PORPHYRINS AND PORPHYRIN EXCRETION IN M. H., ♀ 5, PORPHYRIA ERYTHROPOIETICA

	March 28 to April 4, 1951					June 21, 1951			
						Liver, µg./100 Gm.		Spleen, µg./100 Gm.	
	Erythrocytes, µg./100 Gm.	Serum, µg./100 cc.	Bone Marrow, µg./100 Gm.	Urine, µg./24 Hr.	Feces, µg./24 Hr.	Fresh	Heated*	Fresh	Heated*
Uroporphyrin	280	4.0	2,493	25,200	340	178	869	1,739	3,415
Coproporphyrin	90	19.0	613	2,800	43,440	346	429	821	735
Protoporphyrin	42	0	190	0		45	45	49	104
Porphobilinogen				0				0	

* Fifteen minutes at 80 to 90 C., in a covered beaker to prevent evaporation.

In the cases of erythropoietic porphyria which have come to autopsy, the bones have been characteristically red due to deposition of uroporphyrin (23, 62, 85, 88). In this connection, Fraenkel's (48) demonstration years ago that the injection of uroporphyrin in young animals results in deposition where new bone is being laid down is noteworthy. Together with the fact that uroporphyrin is readily adsorbed and precipitated by calcium phosphate *in vitro* (28, 128), this

porphobilinogen in the urine; these disappeared completely during remissions.

The familial occurrence of the disease is marked (142, 156). Waldenstrom *et al.* (142, 146) regard acute porphyria as dominant in inheritance, in contrast to "porphyria congenita," which is believed to be recessive; this agrees with our observations. There is need, however, of a more detailed genetic study of porphyria in general, based on recent observations on pathogenesis. In many of our cases, in which urine samples from other members of the family could be examined, instances of latent porphyria were detected. Family histories often disclosed that other members had had attacks, sometimes fatal In certain of the latent cases an attack occurred later. In fact, one of our recent patients was suffering from abdominal and leg pain but did not consult a physician until his sister was admitted to the hospital with an ultimately fatal attack of acute porphyria Examination then revealed that the brother and an uncle both had the disease, the latter, however, in purely latent form.

The mechanism by which the latent disease is converted to manifest disease, i e., an attack of acute porphyria, is unknown, but it is increasingly clear that there are various types of precipitating factors. Gunther (61, 62) distinguished between a toxic and an idiopathic acute porphyria, implying that in the former variety factors such as sulfonal and trional were of primary etiologic significance. Subsequently, however, it became well established that certain chemicals, notably barbiturates, can precipitate attacks, at least under certain circumstances in individuals with latent disease, either with or without abnormal urinary findings Waldenstrom (142) was the first to establish beyond doubt the precipitating action of barbiturates in acute porphyria. He administered a barbiturate to a woman who had never had an attack but whose sister had had intermittent acute porphyria. Following ingestion of the drug, an attack occurred for the first time. Jørgensen and With (67) described a similar experiment with the same result. The precipitation of attacks by barbiturates appears to have been observed with increasing frequency. This can probably be ascribed to the more widespread use of these compounds in individuals whose latent disease would otherwise have gone unrecognized It is of interest in this connection that, as Gunther recognized, these patients often exhibit an abnormal nervous behavior for years before the disease becomes manifest Gunther (62) spoke of this as "por-

do not show erythrodontia in ordinary light may exhibit red fluorescence in Wood's light. This was true in both of our cases. The erythrodontia of porphyria is to be distinguished sharply from the less marked red fluorescence due to yeasts or bacteria which may be present on the surface of the teeth and can be removed at least in part.

PORPHYRIA HEPATICA

This is by far the largest and most important group. As can be seen from Table 2, all but 5 of 160 cases were of this type. The relative incidence of the subtypes of the hepatic group may differ, depending on geography or race. Thus, the cutanea tarda type is said to be much more frequent than acute porphyria in Buenos Aires (78), while in the series which we have studied the ratio of acute to cutanea tarda cases was approximately 4:1, or 3:1 if the cases of combined type are included with the cutanea tarda group.

Functional impairment of the liver or outspoken liver disease is often observed in this group Jaundice is seen at times, especially in the cutanea tarda or combined types of cases. Some degree of bromsulfalein retention is present in most cases of the hepatic group and is often marked, despite lack of jaundice. The available evidence permits no conclusion about the exact relationship of the hepatic disturbances to the porphyria Because of the outspoken familial incidence, it seems possible that the liver affection is secondary to the porphyria or that in some cases an intercurrent disease has precipitated active manifestations in an individual with latent porphyria. Brunsting *et al.* (10) have recently emphasized the relative frequency of alcoholism, fatty liver, or fatty cirrhosis in their cases of chronic or cutanea tarda porphyria.

INTERMITTENT ACUTE PORPHYRIA

A marked predilection for the female sex of this form of porphyria, emphasized in a number of reports (23, 62, 85, 142), has not been borne out in our experience, we have found the ratio of female to male to be about 1.5:1 (Table 2) Young adults or the middle-aged are the most frequently affected Acute porphyria is extremely rare in childhood and after the age of 60. Nevertheless, one of our recent cases was that of an 8 year old boy in whom attacks of abdominal pain and constipation were accompanied by the presence of uroporphyrin and

reason, too, to doubt that uroporphyrin or coproporphyrin itself is directly involved in the production of pain Thus, porphyrinemia is much more marked in the erythropoietic and in the hepatic cutanea tarda forms of the disease, in which pain and neurologic disturbances are entirely lacking. In these forms, porphobilinogen is not present in the urine, whereas the attacks of the intermittent acute form are regularly characterized by the presence of large amounts; in some cases only porphobilinogen has been found, without uroporphyrin and indeed without any excess of coproporphyrin. Waldenstrom observed two such instances, in both of which, however, at a later date, uroporphyrin was observed. We (157) have repeatedly studied a woman of 30 who has had no less than 10 laparotomies for abdominal pain of undetermined cause (prior to the eventual diagnosis of porphyria); in this case, despite the presence of considerable amounts of porphobilinogen, no increase of porphyrin has been found. Nevertheless, it may be significant that the serum coproporphyrin in this case, at a time when there was still pain, was 9 µg per 100 cc. Normally, the range is from 0 to 1 µg. It is doubtful, however, that the pain can be ascribed to the porphyrin on this basis, as similar mild degrees of coproporphyrinemia are encountered in a variety of other pathologic states, including hepatic disease, obstructive jaundice (136), and certain neurologic disorders (158) Intravenous administration of coproporphyrin III in amounts ranging up to 5 mg. in a normal volunteer failed to produce symptoms or elevation of blood pressure.*

The hypertension of acute porphyria is especially intriguing and quite unpredictable in occurrence, in some cases being outspoken, in many completely lacking Waldenstrom (142) reported retinal angiospasm in acute porphyria, in association with hypertension. We have seen this repeatedly, one of the most striking examples being associated with repeated convulsions All of these manifestations promptly disappeared as the patient went into remission, possibly as a result of ACTH administration. Electrocardiographic changes believed due to coronary artery spasm have been noted in such cases in association with hypertension (27) Nesbitt (91) pointed out that in some cases the clinical picture more nearly simulates that of Addison's disease, a type that we have seen repeatedly. The question has been raised whether adrenal cortical insufficiency is a factor, so far as the occurrence of hyponatremia, weakness, pigmentation, and coma are con-

* Unpublished observation with Wm Terry

phyrismus" and regarded it as a constitutional feature. In respect to latency, it is not at all uncommon in porphyria families to find that one parent, even though in the sixth or seventh decade of life, has typical urinary features of latent porphyria but has never had active symptoms of the disease.

The varied manifestations of the disease have been adequately described in several relatively recent reports (23, 142, 156, 158) and need not be repeated here in any detail. Several excellent reports deal more particularly with the neurologic and psychiatric features of the disease (19, 50, 119, 143). It is possible, as Waldenstrom has done (142), to distinguish a number of subtypes of acute porphyria, which in many instances are relatively clear cut. These include psychic or hysteroid, peripheral neuropathic or quadriplegic; bulbar or respiratory; convulsive or abdominal. However, the combinations are so many and so varied that these subtypes have relatively little importance. In the abdominal form, the disease often simulates hepatobiliary, renal, or intestinal disease of one type or another, and its importance in surgical diagnosis has been increasingly recognized (11, 103). Acute porphyria has indeed simulated so many diverse conditions that it well deserves the sobriquet of "little simulator" (143). Pain, of course, is the outstanding single symptom, with regard to frequency and the call upon the doctor for relief. The exact mechanism of production of pain, whether in the abdomen or in the extremities, is unknown So far as the abdomen is concerned, there can be little doubt that smooth muscle disturbances, spasm, and distention are the immediate cause of pain Carrié (14) and others have championed the view that the porphyrin itself is spasmogenic This is supported to some extent by the observations of Reitlinger and Klee (104) that very dilute solutions of coproporphyrin placed on a loop of guinea pig intestine caused prolonged tonic spasm, refractory to atropine but relaxed by epinephrine All of this has been amply confirmed by Chadbourn.* Nevertheless, epinephrine has consistently failed to relieve abdominal pain, even when the pain was associated with smooth muscle spasm in the colon, as clearly demonstrated by x-ray. On the other hand, sympathetic ganglion blocking agents often produce dramatic relief, and in 1 case (as discussed on p. 284) splanchnicectomy has had a beneficial effect This suggests an autonomic disturbance rather than a direct smooth muscle effect There is considerable

* Unpublished study, Departments of Pharmacology and Medicine, University of Minnesota.

cannot be differentiated. The addition of a small amount of concentrated hydrochloric acid, however, results in a characteristic porphyrin–HCl spectrum, with a strong absorption band at 553 mμ and a relatively weak one at 597 mμ (only the maximal values are given). Oxyhemoglobin treated in the same fashion yields acid hematin with broad diffuse absorption in the red (630–640 mμ). Smaller amounts of uroporphyrin are also found in the feces, together with increased coproporphyrin, the increase of the latter being mainly of type III isomer.

Until recently, little definite evidence was reported with regard to the site of origin of the porphyrins and their precursors in acute porphyria. Vanotti (138) brought together some observations of his own and of others suggesting a primary role of the liver. Waldenström (142) was impressed with the importance of disturbed liver function in the genesis of acute porphyria. Schmidt (119) has recently reviewed a number of reports relating to this question. Turner (132), in 1940, in proposing an interesting theory of porphyrinogenesis which has proved to be very much along the lines of recent suggestions, also made the following rather prophetic statement: "Repeatedly, fluorescence characteristic of the three types of porphyrins [proto-, copro-, and uroporphyrin] has been observed in erythroblasts, gradually fading as the addition of iron and protein dampened it. *However, in porphyria, the frequent demonstration of zinc uroporphyrin, as well as of other porphyrins in the peripheral portion of liver lobules, suggests the possibility that in this condition cells other than erythropoietic may be active in porphyrinogenesis*" Others (138) have found no increase of porphyrin in the livers of cases that have come to autopsy. Prunty (100) reported the presence of considerable amounts of porphobilinogen in the liver in such a case, and described a method of extracting and concentrating it He drew no conclusions as to site of formation The probable relation of the liver to excessive porphobilinogen and porphyrin formation is discussed by Schmid and associates (115). Schmid's studies were the first to compare the content in bone marrow, liver, and other viscera of porphyrins and porphobilinogen in this as well as the other forms of porphyria In striking contrast to the "congenital" or erythropoietic type, as already discussed, the bone marrow and its porphyrin content was found to be normal in the intermittent acute form. The liver, on the contrary, regularly exhibited increases of porphyrin, much or all of which, however, was represented as nonfluorescing precursor or chromogen. When sufficient liver was available to permit testing with Ehrlich's

cerned. This was suggested in an unusual case reported by Abrahams *et al.* (1), and subsequently by others (18, 76) in similar instances Prunty (102), however, was unable to find objective evidence of adrenal cortical hypofunction either in life or at necropsy in cases of this type Our own experience indicates that the patients who are profoundly weak or comatose usually have electrolyte disturbances related to vomiting, dehydration, and inanition.

The urine in the acute form of porphyria characteristically contains moderate to marked excesses of uro- and coproporphyrin and porphobilinogen, which increase during relapse and decrease during remission. Characteristic data for porphobilinogen are given in Table 4. The Waldenström uroporphyrin, which is characteristically present, is excreted mainly or entirely as a metal complex (20, 21, 147)

TABLE 4 —URINARY PORPHOBILINOGEN IN RELAPSE AND REMISSION OF ACUTE PORPHYRIA

CASE	RELAPSE*	REMISSION
a	++	+
b	45 units/day	12 units/day
c	56 units/day	23 units/day
d	++	0–2 units/day
e	51 units/day	0–2 5 units/day
f	40 units/day	8 units/day
g	+	negative
h	+++	negative

* + – ++ + refers to qualitative tests, on a + (weak) to ++++ (intense) basis

This has been crystallized, with identification of the metal as zinc (57, 150).

The significance of the zinc complex is not known Derrien and co-workers (20, 21) believed there might be a zinc deficiency, but Nesbitt (91) found normal zinc content of liver and pancreas in a fatal case. In erythropoietic porphyria a great majority of the porphyrin is excreted in the free state This suggests that during porphyrin formation in the developing red cells zinc is not as available for complexing, perhaps because of lower concentration in the marrow and a greater demand for zinc in leukocytes and in the fabrication of carbonic anhydrase. Conversely, it might reasonably be considered that the rich hepatic store of zinc would be more readily available for complexing with porphyrins or their precursors, formed in the liver. Other possibilities, however, have not been excluded.

The absorption spectrum of the zinc complex is essentially similar to that of oxyhemoglobin, in fact, with an ordinary spectroscope, they

in one of our cases and in the case recently reported by Lyons (81). It seems not unlikely that the occasional appearance of porphyrin in the cerebrospinal fluid is analogous to that of bilirubin in patients with jaundice. Both may depend on a disturbance of the blood-brain barrier incident to liver injury Thus, Amatuzio and associates (3) have shown that the appearance and concentration of bilirubin in the cerebrospinal fluid depend more on hepatic insufficiency than on the level of serum bilirubin

Moderate increases of proto- and coproporphyrin have been found in the bile in some cases, but porphobilinogen has not been detected with certainty In the first case studied at autopsy in this laboratory, in 1938, the cirrhotic liver contained large amounts of a uroporphyrin zinc complex, this was also found in the feces in smaller concentration, but gallstones and bile from the gallbladder yielded only minute amounts of coproporphyrin. At that time, the significance of porphobilinogen was unknown and it was not sought for In a case recently observed at autopsy, the liver contained porphobilinogen in considerable amount, the Ehrlich reaction on the bile was positive, but since most of the aldehyde compound was extractable by chloroform it was believed to be urobilinogen, and whether any porphobilinogen was present was uncertain These findings are of special interest in relation to the obvious excesses of porphyrin and porphobilinogen in the bile of rabbits with Sedormid porphyria

Cutanea Tarda Type

This term was used by Waldenstrom (142) to designate the "chronic" cases of Gunther's classification (62). Waldenstrom's designation is preferable and perhaps best expresses the most prominent feature of the disease—the late appearance of photosensitivity and cutaneous lesions Although it is true that this form is very chronic, chronicity also characterizes the erythropoietic group, and indeed some of the intermittent acute cases as well Thus the terms "chronic" and "congenital" are believed unsuitable.

In most cases in this group, photosensitivity first develops after the third or fourth decade of life In an occasional individual, skin lesions appear relatively early, and they have even been noted in children. In 1 of our patients in this group, symptoms first appeared at the age of 5 Careful study in this instance failed to reveal any evidence that the disease was related to erythropoiesis

reagent, it was possible to detect porphobilinogen. In the cases in which liver biopsy material alone was available, the demonstration of porphobilinogen or precursor depended upon quantitative determination of uroporphyrin before and after heating, in some instances, no uroporphyrin could be demonstrated prior to heating (Table 5).

Fluorescence microscopy and chemical assay of other tissues including the kidneys, spleen, and nervous system have often revealed

TABLE 5 —UROPORPHYRIN CONCENTRATION IN LIVER AND URINE BEFORE AND AFTER CONVERSION OF PRECURSOR (115)

CASE No *	LIVER, µG /100 GM (WET WEIGHT)		URINE, µG /100 ML	
	Before Heating	After Heating†	Before Heating	After Heating†
76	178	869	15,150	17,050
42	73	133	270	1,500
66	23	804	32	2,080
92	trace	445	120	7,840
117	70	410	5,180	32,000
138	—	1,604	113	15,800
137	18	143	595	1,425
103	2,950	540	1,920	2,430
115	12,000	—	420	1,360
118	3,360	2,835	—	—
86b	31	528	305	3,980
55	47	131	—	—
60	1,080	3,390	—	—

* Case 76 erythropoietic porphyria Cases 42 55, 60, 66, 92, 117, 137, 138 intermittent acute porphyria (hepatic) Cases 103, 115, 118 cutanea tarda porphyria Case 86b. combined hepatic porphyria during period of abdominal pain and hysteroid behavior, without photosensitivity
† Adjusted to pH 4 to 5 and heated for 20 minutes in boiling water bath

the presence of smaller amounts of porphyrin, but porphobilinogen has been demonstrable only in the liver (115). In accordance with Kluver's earlier studies (72), the nervous system in acute porphyria often shows a patchy porphyrinization, more particularly of certain of the cranial and peripheral nerves The content of porphyrin in the central nervous system has been unimpressive. Porphobilinogen has not been demonstrated The cerebrospinal fluid, as a rule, fails to reveal porphyrin or porphobilinogen There are certain exceptions, as

one period, with minimal abdominal and nervous symptoms, a year later, the reverse of this situation was found. It was believed important that the liver during the period of photosensitivity contained mainly preformed porphyrin, fluorescing strongly in the fresh unheated state. In the later period, however, most of the abnormal pyrrol compound represented in the liver was in the form of nonfluorescing precursor (probably porphobilinogen), first converted to porphyrin on heating the liver emulsion (115). This is in accord with Schmid's findings in other cases of hepatic porphyria, i.e., that in the intermittent acute group most of the porphyrin is present as precursor, while in the cutanea tarda cases large amounts of preformed porphyrin are readily demonstrable in the fresh liver (115). These findings, and particularly the alternation in the ratio of precursor to preformed porphyrin in the same case at different times, suggest a basic relationship between the intermittent acute and the cutanea tarda types, with the combined types representing a transition. In reviewing the literature, one is impressed by the many and varied combinations that have been described. Nesbitt and Watkins (90) emphasized this point in describing a case of acute porphyria with photosensitivity. Garcin and Lapresle (50) recently referred to an instance of fatal (nervous) porphyria in which photosensitivity had been present in earlier years Waldenstrom (142) failed to observe this in any of his series of cases in the acute category; this divergence may be due to the fact that no criteria have been provided for a sharp dividing line between cutanea tarda cases with abdominal and those with nervous manifestations. The concept of a fundamental relationship with the acute disease is also supported by the occurrence of families in which one member has acute porphyria, another only photosensitivity or cutanea tarda manifestations (12, 74, 133). We have had the privilege of studying* a member of such a family reported by Calvy *et al* (12) This study, carried out over a protracted period, had special reference to porphyrin chemistry and biosynthesis (79) The patient, a 21 year old man, had had intermittent photosensitivity since age 16, both in association with, and independently of, abdominal pain and hysteroid behavior.

Rimington and co-workers (82, 108) regard the cutanea tarda and intermittent acute cases as fundamentally distinct entities, and would classify the mixed, or combined, form with the cutanea tarda, and

* Through the courtesy of Dr George Calvy

The skin lesions closely resemble those of the erythropoietic form. The mutilation, as a rule, seems not as great as in the adult phase of the latter, which may simply be due to the long duration of the disease. However, available evidence indicates that the porphyrinemia in the cutanea tarda type is not as marked as in the erythropoietic form. Furthermore, the uroporphyrin in the latter consists almost wholly of the type I isomer, whereas in the cutanea tarda form a Waldenstrom type of complex (preponderantly type I, with some type III) is found. It is worth emphasizing again that in patients with cutanea tarda porphyria the porphyrin content of the bone marrow is normal, in striking contrast to that of the erythropoietic type

In addition to the straightforward photosensitivity, some of these cases exhibit skin lesions of epidermolysis bullosa type, not necessarily on light-exposed surfaces of skin (10, 131). Heat as well as trauma have been noted to produce bullae (10). Brownish pigmentation of the skin is not uncommon, at times assuming a violaceous or purplish hue. The latter characteristic was especially frequent in the cases observed by Brunsting *et al.* (10). We, too, have seen it on occasion (158). Melanosis and changes in hair color, especially from red to black may occur (10).

Manifest liver disease is often present in this group, although in many instances a thorough study of liver function is necessary to uncover evidence of impairment. Significant bromsulfalein retention is usually encountered. A fair number of these patients have definite hepatic cirrhosis, with or without jaundice or ascites. Others have fatty liver. Alcoholism has been unusually frequent in the cutanea tarda cases as a whole (10) It seems not unlikely that this may play only a precipitating role, much as do the barbiturates or other chemicals, including alcohol, in the intermittent acute cases

Mixed or Combined Type

As the name implies, this form embraces cutaneous, abdominal, and/or nervous manifestations. The latter may or may not be concurrent with the photosensitivity Excesses of porphyrin or porphyrin precursors are readily demonstrable in the livers of these patients, while the porphyrin content of bone marrow and red cells is normal or not more than slightly increased to an extent which is probably related to the mild increases in the circulating plasma (115).

In one of our cases (115), photosensitivity was prominent during

likely that there was any considerable increase. Furthermore, methods now in use for tissue porphyrin assay (115) were not available then. Neither of these cases revealed any evidence of blood dyscrasia. The erythrocyte porphyrin content was normal in both. Large amounts of coproporphyrin were found in the bile and feces. It seems reasonable to assume that these cases may represent variants of porphyria hepatica in which, for reasons unknown, the abnormal pyrrol metabolism is limited to excessive coproporphyrin formation. The coproporphyrin excretion in the feces ranged from 30 to 70 mg. per day, and in the urine from 1 to 10 mg. (Table 6). No significant excesses of protoporphyrin were encountered in the feces. This is also of interest because in some cases of porphyria, the amount of protoporphyrin in the feces is markedly increased (79, 82). In one of our cases, best classified as a combined type of hepatic porphyria, the feces contained relatively large amounts of copro- and protoporphyrin, with very small amounts of uroporphyrin (79). The urine in this case contained mainly coproporphyrin III with small but distinct increases of a Waldenstrom uro-type porphyrin.

It is possible that the 2 cases of idiopathic coproporphyrinuria just referred to would under certain conditions exhibit excesses of uro- and/or protoporphyrin, and the possibility of the appearance of symptoms is, of course, not excluded. A few other cases have been labeled as porphyria, in which uroporphyrin was not demonstrated Kammerer and Meyer (68) described a case of "abdominal idiopathic" porphyria in which excessive coproporphyrin was excreted, without uroporphyrin. Interestingly, however, the urine gave a strong Ehrlich reaction, in retrospect probably due to porphobilinogen These authors concluded that the excessive coproporphyrin was derived from dietary hemoglobin protoporphyrin, believing that this was absorbed and converted by the liver to coproporphyrin, in accordance with certain experimental results of van den Bergh *et al.* (136). More recent work (75, 110, 148) has failed to confirm these concepts. Although proto- and coproporphyrin may well be formed by bacterial activity in the colon both from hemoglobin and as independent metabolites, recent evidence (75) indicates that these do not affect the internal porphyrin metabolism nor the urinary porphyrin excretion. Furthermore, it is not clear whether the presence of uroporphyrin was excluded with certainty in Kammerer and Meyer's case. Van den Bergh and Grotepass (137) described an interesting example of "chronic" (cutanea tarda) porphyria in which there was "porphyrinemia without porphy-

quite separate from the acute. Rimington places special emphasis on his belief that the feces, during remission, contain much more porphyrin in the cutanea tarda than in intermittent acute cases. In our own experience, this difference has not been decisive. One must also take into account the fact that in the acute type a considerable portion, even the largest part of the abnormal pyrrole formation, is represented by porphobilinogen which is excreted only in the urine, while in the cutanea tarda type porphobilinogen is not demonstrable and larger proportions of the copro- and all of the protoporphyrin excreted are to be found in the feces. Nicholas and Rimington (95) also place considerable stress on recent studies of the composition of the Waldenstrom porphyrin in the two forms. This is referred to in more detail in the following

TABLE 6.—PORPHYRIN EXCRETION IN TWO CASES OF IDIOPATHIC COPROPORPHYRINURIA (159)

Case No	Urine, µg /24 Hr	Feces, Mg /24 Hr		Duodenal Contents µg /100 Cc
	Coproporphyrin	Coproporphyrin	Protoporphyrin	Coproporphyrin
1	1,200–6,000 (86–95% III)	32–69 (mainly III)	0 38–1 2	308–1,327
2	1,400–8,000 (98–100% III)	32 8–40 2 (90% III)	—	128

IDIOPATHIC COPROPORPHYRINURIA.—It is desirable to consider briefly a rare condition in which large amounts of coproporphyrin are formed and excreted in the feces and urine without any demonstrable increase of uroporphyrin or porphobilinogen formation or excretion. Two such cases, both entirely symptomless, have been reported (152). One patient was a chemist who had had ordinary chemical exposures in the laboratory, he felt entirely well, and careful examination revealed no objective evidence of any disease The other was a Negro admitted to the hospital because of diphtheria, antitoxin therapy led to uneventful recovery in a few days, but coproporphyrin excretion continued to be excessive, although without symptoms, long after he recovered The magnitude of the porphyrin excretion is shown by the data in Table 6

At the time these observations were made, relatively sensitive technics for the detection and quantitation of uroporphyrin in urine and feces were not available. None at least could be detected and it is un-

isomer the correct melting point was 311 C. Grinstein and associates (57, 150) later noted, however, that this melting point was much too high, due principally to inclusion of metal complex. The metal complexes of the porphyrin esters regularly exhibit higher melting points than the free esters. These workers also pointed out that the inclusion of any unesterified or free porphyrin with the ester elevates the melting point Grinstein *et al.* reported that the melting point of uroporphyrin I methyl ester was usually 284 to 286 C., but observed samples which had lower or higher melting points even after repeated calcium carbonate chromatography and recrystallization (57). Nicholas and Rimington (95) have recently stated that the true melting point is 293 C., the same as that given originally by Fischer. It may be noted that the melting point of this compound is unusually difficult to determine as the microscopic crystals are long and hairlike, wavy and intertwining, forming fluffy masses. Even with the purest of material the melting point is not very sharp, considerable charring and shrinking being noted prior to liquefaction. It is difficult to maintain the methyl ester completely free of saponified material, or free porphyrin, small amounts of which elevate the melting point. Nicholas and Rimington (95) have given a melting point curve for varying mixtures of uro-I (erythropoietic porphyria) and uro- III (turacin). The application of the curve to any naturally occurring uroporphyrin mixtures obviously requires, however, that they be shown to consist solely of these two porphyrins, without inclusion of any other uro-type porphyrins, using this latter designation to include ether-insoluble porphyrins with spectra similar to that of uroporphyrin I, whether octacarboxyl or not (The term "uroporphyrin" in the truest sense requires 4 acetic and 4 propionic acid substituents.) Thus it would be anticipated that the curve would not be applicable if the mixture contained even very small proportions of the 208 porphyrin (see below) or one of the less well-defined octacarboxyl porphyrins recently described by Rimington, such as that obtained from turacin (m p 210 C) (92), and those from congenital porphyria urine (m p 234 and 244 C, respectively), and regarded by Rimington (107) as associations of uro- I and III; also similar, but as yet poorly defined, uro-type porphyrins found in experimental (Sedormid) rabbit porphyria urine.

Uroporphyrin I has been shown to have marked photodynamic activity (8, 34), and there is every reason to believe that it is responsible for the photocutaneous lesions of the erythropoietic form of porphyria. Yet it has not been possible to reproduce skin lesions by

rinuria." In this case, a careful search for uroporphyrin in urine, blood serum, bile, and feces was fruitless. The serum, bile, and feces contained large amounts of coproporphyrin. That isolated from the bile was identified as type I, the hemoglobin protoporphyrin in this case being shown to be the normal type 9 (isomer series III). Similar cases were studied by Dobriner (22) and by Taylor *et al.* (130), in which both coproporphyrin isomers (I and III) were found in the urine.

Several other cases have been observed in which there were mild increases of coproporphyrin in the urine in association with abdominal pain or mild neuritic manifestations. The significance of these is not at all clear. It is entirely possible that they represent unrecognized chemical toxicity in which the increase of urinary porphyrin is clearly secondary in character (151). For example, we have observed a case characterized by hysteroid behavior, pain in the extremities, and persistent mild increase of urinary coproporphyrin III over a long period, in which it was eventually disclosed that the patient was a barbiturate addict. The importance of coproporphyrinuria in relation to chemical and heavy metal poisoning has been discussed in a number of reviews (23, 138, 151, 156).

BIOCHEMICAL VARIATIONS AND PATHOGENESIS OF MANIFESTATIONS

In Porphyria Erythropoietica

As already mentioned, most of the copro- and uroporphyrin excreted in this form of the disease is the type I isomer Fischer's early reports (31–33, 37) indicated that it was entirely type I. Some years later, on re-examining the uroporphyrin from case Petry, by Al_2O_3 chromatography, Fischer *et al.* (46, 47) reported the presence of a small proportion of type III isomer. The melting point of uroporphyrin I methyl ester has been variously given Dobriner and Rhoads (23) reviewed the earlier data, including Dobriner's own. Fischer (31) originally recorded it as 293 C Other values, as given by Dobriner and Rhoads, ranged from 272 to 302 C Dobriner himself observed it to be 279, 285 and 286 C in three different cases Fink and Hoerburger (30) reported a melting point of 293 C for crystals isolated from the bones of porphyric cattle Rimington (105), with similar material, noted melting points of 273 to 277 C, the crystalline uro- ester from the cattle urine melting at 277 C Subsequently, Fischer and Hofmann (46) reported that after removal of small amounts of type III

and Duesberg (42) obtained only coproporphyrin I on decarboxylating a uroporphyrin from acute porphyria urine, the methyl ester of which melted at 269 C. Grinstein and associates (57) later reported that calcium carbonate chromatography of the porphyrin mixture resulting from decarboxylation of the Waldenstrom porphyrin yielded, in addition to coproporphyrin, a pentacarboxyl porphyrin, together with smaller amounts of other unidentified porphyrins The primary decarboxylation mixture melted at 160 to 162 C, a melting point which could readily be confused with that of copro- III methyl ester Calcium carbonate chromatography of the Waldenstrom porphyrin itself often permitted a separation into two distinct zones (57) The upper zone, comprising the larger portion, melted at 284 C., and on decarboxylation yielded coproporphyrin I with varying amounts of a pentamethyl ester melting at 224 C, on further decarboxylation, the latter also yielded only coproporphyrin I The lower zone melted at 208 to 210 C., and on decarboxylation yielded coproporphyrin III, and at times a small amount of a methyl ester melting at 183 C, having a position on the column corresponding with a pentamethyl ester. As a result of this work it was concluded that the Waldenstrom porphyrin was a molecular complex of uroporphyrin I with a smaller amount of a type III porphyrin having a lesser number of carboxyl groups Methoxy determination indicated that the latter was a heptacarboxyl porphyrin Watson and co-workers (150), following upon the study of Grinstein *et al*, noted that in many instances the Waldenstrom porphyrin from acute porphyria urine behaved as an entity on the calcium carbonate column, i e, it could not be separated into two zones as just noted, and upon recrystallization of the single zone, the melting point was unchanged. This was designated as a type B Waldenstrom porphyrin, in contradistinction to type A, which separates on $CaCO_3$. Nevertheless, on decarboxylation of this porphyrin, followed by chromatography and fractional crystallization of the copro-porphyrin esters, the major portion regularly showed the melting point of coproporphyrin I (245–250 C) While this work did not exclude the presence of uroporphyrin III in molecular combination with the type I isomer, evidence for its presence was not obtained Subsequently, the finding that the major component of the Waldenstrom porphyrin was the type I isomer was confirmed by Prunty (101), Gibson and Harrison (52), and Gray (56) McSwiney *et al* (85) later reported that "additional confirmation has been obtained of the fact that the uroporphyrin of acute porphyria is mainly a type I

local exposure to light of the same wavelengths as that principally absorbed by the uroporphyrin (6, 7, 23). Two possibilities must be considered in this connection: (1) The light exposure has simply been inadequate, as contrasted with repeated or intensive exposure to sunlight. Blum and Pace (7) state, however, that hydroa was not produced even with sufficient radiation to produce sunburn in normal human skin (2) Any considerable exposure to sunlight results in an overproduction of porphyrin, and in turn either porphyrinemia or an increased porphyrinemia. This is hypothetical but is strongly suggested by the history in some cases that the urine became red or more intensely colored following exposure to sunlight in the spring or early summer (4, 115); also, the recent observations of Pimenta de Mello (97) that the photodynamic effect of rose bengal in rabbits is attended by a marked, though transitory, coproporphyrinuria He also noted that the coproporphyrinuria of lead poisoning in rabbits is enhanced by exposure to light (98) This whole question invites further study.

Porphyrins and Pyrrol Compounds In Porphyria Hepatica

Prior to Waldenstrom's studies of the uroporphyrin of acute porphyria urine, the methyl esters of the uro-type porphyrin which had been isolated had been noted to exhibit melting points considerably lower than in the "congenital" cases. Earlier data collected by Waldenstrom and co-workers (141), in 1935, showed a range of 257 to 274 C. The first detailed study of this porphyrin was made by Waldenström and associates (140–142) Waldenstrom observed that in contradistinction to uroporphyrin I, it is extractable from the urine by ethyl acetate at pH 3 2 (gray-blue reaction to Congo paper). The melting point of the crystalline methyl ester was usually between 258 and 260 C., at times, however, higher or lower. This was confirmed by Mertens (86) Both investigators reported the isolation of coproporphyrin III following decarboxylation It should be noted however, that the decarboxylation mixture was not submitted to chromatographic analysis, which at that time was relatively little used in porphyrin work.

At about the same time, Fischer and Libowitzky (44) isolated uroporphyrin I from the urine of a case of acute porphyria (m.p 279–280 C. decarboxylation → copro- I) Mertens obtained coproporphyrin I from a sample of uroporphyrin provided by Rimington, isolated from acute porphyria urine and melting at 268 C. (105). Similarly, Fischer

adequate evidence that the porphyrin being compared by the methods just referred to is composed solely of uroporphyrins in the truest sense. Nicholas and Rimington (95) regard the 208 porphyrin, mentioned earlier as a type III component of the Waldenström complex, as an insignificant trace which they have not themselves encountered using the magnesium oxide method. More recent studies in this laboratory, however, have consistently shown that when a type A Waldenstrom porphyrin has been isolated, its separation on a $CaCO_3$ column provides from 15 to 30 per cent of the type III 208 porphyrin ester. Further methoxy determinations have consistently indicated a 7-COOH ester.

Professor Rimington kindly undertook a study of some type A Waldenstrom porphyrin as isolated in this laboratory and found by us to separate on $CaCO_3$ into uro- I and the 208 porphyrin By ordinary paper chromatography it behaved mainly as an 8-COOH, a very small fraction having an Rf comparable to 6-COOH. We also submitted some 284 or uro- I ester obtained from a type A porphyrin on $CaCO_3$ He readily confirmed that this was uro- I by Falk-Benson chromatography and x-ray crystallography

Studies to be described elsewhere have shown that the type A Waldenstrom porphyrin, while often behaving in similar fashion to the turacin uro- III when examined by the Falk-Benson method, nevertheless yields a majority of uro- I on $CaCO_3$. It should be noted in this connection that many samples of $CaCO_3$ are unsatisfactory and fail to separate the I and III components. Methods of preparation and characterization of suitable $CaCO_3$ will be described elsewhere. A direct comparison of $CaCO_3$ and MgO with a number of samples of type A porphyrins has failed to reveal separation into the I and III components with MgO in any instance. This refers to the flowing technic used by Nicholas (93). Thus it is difficult to understand how the 208 porphyrin would be detected or removed by this method. To what extent it would interfere in interpretation of mixed melting points, x-ray crystal powder patterns or infrared spectra remains to be determined.

The Waldenstrom porphyrin studied by Rimington has been isolated by a method different from Waldenström's, one involving in most instances preliminary heating of the urine until the Ehrlich reaction for [illegible]

Gru[illegible]

rela[illegible]

isomer." Gibson and Harrison made the interesting observation that the fresh urine in one case contained uroporphyrin I, the methyl ester melting at 280 C., whereas after boiling the urine, and associated with a very great increase in concentration of uro-type porphyrins, the porphyrin methyl ester obtained melted at 260 C. and behaved like a type B Waldenstrom porphyrin. On decarboxylation, however, coproporphyrin I was isolated. This is an example of the unreliability of the melting point alone as a safe distinguishing feature or as a guide to the character of the molecular complex. Many additional examples might be cited both from the literature and from our own experience In the recent report of Canivet *et al.* (13), the presumed identification of the uroporphyrin as type III, in 9 cases of acute porphyria, was based principally on the melting point, which ranged from 253 to 261 C. Quite recently Paul and co-workers (96a) reported isolation of a uroporphyrin from the bones of a case of porphyria cutanea tarda. It was concluded that this was uroporphyrin III solely on the basis of the melting point of 264 C. Nicholas and Rimington (95) have emphasized that this is unreliable.

Rimington and associates (29, 71, 82, 95, 106) have published data indicating that the Waldenström porphyrin in the urine of patients with acute porphyria is composed of about 75 per cent uroporphyrin III and 25 per cent uroporphyrin I. For reference they used artificial mixtures of pure uroporphyrin I from erythropoietic, or congenital, porphyria urine, and uroporphyrin III, as obtained from turacin. The methods employed for this comparison were: (*1*) the Falk and Benson paper chromatographic technic (29), which permits the separation of these two porphyrins by means of the successive use of two solvent pairs (chloroform-kerosene, dioxane-kerosene); (*2*) Debye-Scheerer x-ray crystal powder pattern (71), (*3*) infrared spectrophotometry (95); (*4*) a mixed melting point curve (95). As emphasized in the foregoing, the translation of results obtained with these methods on pure uroporphyrin I and III to the Waldenström porphyrin complex, as obtained from acute porphyria urine, requires the assumption that the latter is a simple mixture of these two isomeric octacarboxyl porphyrins. Rimington and co-workers (95, 108) now agree that the Waldenström porphyrin is not solely uroporphyrin III as originally believed, but a complex of uro- III (75 per cent) and I (25 per cent). They believe that use of a flowing type of column chromatography with magnesium oxide (93) and paper chromatography, both of ordinary type (94) and according to Falk and Benson (29), provide

the estimation of the variable loss during decarboxylation and the *necessary assumption that this affected the two isomers equally.* Rimington (106) noted the poor yields of coproporphyrin commonly obtained by Fischer's method of decarboxylation. In our recent studies of the Waldenstrom porphyrin, we used a modification of the Fischer method, as devised by Edmondson and Schwartz (26) With samples up to 5 mg, the yield of coproporphyrin is almost quantitative Studies with this method, to be described elsewhere, have confirmed our earlier observations on the preponderance of type I isomer in both forms. Of special interest has been the re-examination of the uroporphyrin ester isolated by Gray (56) from a case of acute porphyria, and which he found to be mainly type I. Rimington (108a) restudied this porphyrin with the methods just referred to and concluded that it was approximately 75 per cent type III and 25 per cent type I. He kindly sent us 11 mg. for comparison Our study showed that while by the Falk-Benson method the porphyrin seemed mainly type III, decarboxylation by the Edmondson-Schwartz method revealed a significant preponderance of type I coproporphyrin, this was confirmed by various methods, including infrared spectrum Nicholas and Rimington (95), in attempting to determine the isomer ratio after decarboxylation, have placed considerable reliance on the Jope and O'Brien (66a) mixed melting point curve of coproporphyrin esters I and III Again, this assumes the sole presence of the two isomers, and does not allow for the possible presence of other porphyrins or impurities which could be expected to affect the melting point.

The complex problem of the Waldenstrom porphyrin has an im- [illegible]

[illegible] lieving that it is formed entirely in the urine, from porphobilinogen, and that the latter is the only substance of any importance in acute porphyria. This will be considered in the following discussion.

Porphobilinogen

The Ehrlich aldehyde reacting chromogen of acute porphyria urine was first distinguished from urobilinogen by Sachs (109), who noted that urines containing the pigment which results from oxidation of this chromogen, unlike urobilin-containing urine, did not exhibit green fluorescence with zinc Later, Waldenstrom and Vahlquist (142,144)

most part at 4 C. It is important to note that in this study nothing was added to the urine during this period. As noted earlier, Waldenstrom extracted the porphyrin from acute porphyria urine by ethyl acetate at pH 3.2 (gray-blue to Congo paper). This method and also one of talc adsorption were used in this laboratory in the earlier studies (57, 150). Reference has already been made to the effect of heating, as observed by Gibson and Harrison (52). Our own observations in recent years have consistently been in accord with this. The Waldenström complex obtained by the talc adsorption method (57, 82, 94), after heating the urine, is regularly type B, i e, an entity on $CaCO_3$. The ethyl acetate extraction method applied to unheated urine usually yields a type A complex, and decarboxylation in our hands has regularly been productive of a preponderance of type I coproporphyrin It is believed significant that this has been true even when, with the Falk-Benson method, the starting Waldenstrom uro- complex has behaved similarly to uroporphyrin III from turacin. In other words, it appears now that this method in many instances does not distinguish uroporphyrin associations, preponderantly type I, from the turacin uro- III. In a recent study to be described elsewhere it has been found that an artificial mixture of 80 per cent uro- I and 20 per cent of the 208 porphyrin esters in chloroform are readily separable by Falk-Benson chromatography, but if the mixture is saponified, dissolved in dilute sodium hydroxide solution, mixed with a large volume of freshly passed normal urine, and allowed to stand over night at 4 C, most of the porphyrin is extractable by ethyl acetate at pH 3 2 By contrast, uroporphyrin I is extracted by ethyl acetate only in very small amounts The association thus formed behaves as an entity on MgO chromatography, m p 258–260 C Of most significance, however, was the behavior on Falk-Benson chromatography, in which it ran entirely as a type III uroporphyrin Decarboxylation, on the other hand, yielded the expected preponderance of coproporphyrin I.

Rimington believes that our finding (57, 150) of a preponderance of coproporphyrin I on decarboxylation of the Waldenstrom porphyrin, and the similar observations of other workers (52, 56, 101), have failed to provide an accurate concept of the amount of type III isomer represented, due to the much greater solubility of coproporphyrin III methyl ester in methyl alcohol. Careful account of this difference must, of course, be taken, and, indeed, it is essential that the amount of uroporphyrin decarboxylated be accounted for quantitatively. This was not done in the past, so that one of the difficulties was

chromatographic technic, the methyl ester is indistinguishable from that of uroporphyrin III obtained from turacin (17, 161), yet it is clear that the decarboxylation mixture is more complex than can be explained if the uroporphyrin were identical with the turacin uroporphyrin III The coproporphyrin obtained by decarboxylation is readily crystallized as the methyl ester from chloroform-methyl alcohol. Cook-

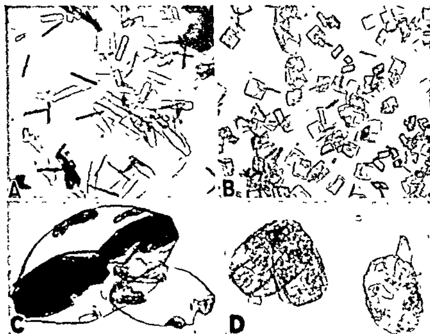


FIG 2 – Porphobilinogen crystals *A*, crystals, ×290 (163). *B*, crystals obtained in the author's laboratory, first separation, ×260 *C* and *D*, crystals obtained by recrystallization of different batches

son and Rimington (17a) observed some melting from 132 to 145 C, a considerable proportion remaining solid to 160 C, and a minority up to 190 C. In our experience, the crystals from methyl alcohol have an indefinite broad melting point, with shrinking above 180 C and complete liquefaction between 210 and 215 C Like coproporphyrin III methyl ester, this porphyrin ester is relatively very soluble in methyl alcohol, quite different from what we have usually observed in the case of the coproporphyrin mixture from the Waldenstrom ester isolated from acute porphyria urine. Recrystallized from ben-

studied the chromogen in greater detail; they recognized its rather general insolubility in organic solvents and pointed out that the Ehrlich aldehyde compound, in addition to the main absorption band (540–570 mμ), also had a weaker band from 515 to 530 mμ. They noted that on brief heating at a weakly alkaline reaction the color was essentially unchanged but the Ehrlich reaction now exhibited only the single band between 540 and 570 mμ. (We have not as yet observed this change, but it is possible that the conditions have differed significantly.) Heating of acid solutions, however, resulted in formation of porphyrin, together with a dark brown, or reddish-brown, nonporphyrin pigment. The chromogen was named porphobilinogen to indicate its relation to porphyrin, on the one hand, and to the nonporphyrin pigment, porphobilin, on the other.

Until rather recently, porphobilinogen has defied all efforts at crystallization Westall (163) achieved this difficult task by a method in which the porphobilinogen is first precipitated from the urine by mercuric acetate, freed from the mercury by H_2S, adsorbed on an ion exchange resin, eluted with ammonium acetate solution, again precipitated by mercury and liberated by H_2S, followed by adsorption on alumina and elution with dilute ammonia. On acidification with acetic acid, porphobilinogen, if sufficiently concentrated, separates from the solution in short rectangular prisms or in larger cake-shaped plates (Fig. 2). Recrystallization is effected by re-solution in ammonia and acidification and chilling The volume of solution must be kept quite small We have found it advantageous to lyophilize the final ammoniacal solution and redissolve in a small volume, prior to acidification. Very recently, Cookson and Rimington (17a) have described a simpler method depending mainly on mercury precipitation and permitting avoidance of the tedious adsorptions on resin and alumina Westall mentioned that a crystalline hydrochloride is readily prepared, and Kennard (70) has described a crystalline hydrobromide The crystalline free substance decomposes at temperatures above 170 C., with simultaneous porphyrin formation A uroporphyrin is also formed on heating the acid solution of the crystalline porphobilinogen. Westall showed that up to 40 per cent conversion occurred on heating for 30 minutes at 80 C., in 0 5 N hydrochloric acid. The exact nature of the uroporphyrin thus formed has not been determined with certainty. Rimington and co-workers have recently reported a melting point of 255 C. We have repeatedly isolated small amounts of this porphyrin with melting points between 254 and 257 C. With the Falk-Benson

x-ray crystallographic data obtained with three types of crystals· the free substance, the hydrochloride, and the hydrobromide Cookson and Rimington have provided ample evidence for the structural formula shown in Figure 3. Also shown in this figure is Cookson and Rimington's concept of porphyrin formation postulated in accordance with the work of Shemin and co-workers (123, 124), which demonstrated that the pyrrol precursor in porphyrin biosynthesis is derived from one molecule of glycine and two molecules of a succinyl derivative, the latter arising in the tricarboxylic acid cycle Shemin (124a), more recently, has shown that the significant intermediary in porphobilinogen biosynthesis is δ-aminolevulinic acid, $NH_2CH_2COCH_2CH_2$-COOH, two molecules of which are utilized in the formation of porphobilinogen. This has been confirmed by Neuberger and Scott (91a)

About a year before Westall's achievement in crystallizing porphobilinogen, it was reported from this laboratory (64) that porphobilinogen solutions could be prepared from acute porphyria urine, which on heating at weakly acid reaction according to Waldenstrom's method failed to exhibit any porphyrin formation. It had previously been noted (154, 155) that an Ehrlich-negative porphyrin precursor or chromogen could often be demonstrated in such urines by extraction with ethyl acetate. If the native porphyrin was then first removed from the ethyl acetate solution by exhaustive HCl extraction, the formation of additional porphyrin was usually demonstrable on relatively brief exposure to near ultraviolet light It was found later that if acute porphyria urine, adjusted to pH 6 0 with acetic acid, was run through a column of aluminum oxide (Brockmann type [Merck], first washed with 1 per cent acetic acid until the eluate was acid), both porphobilinogen and the Ehrlich-negative chromogen were adsorbed and could be eluted slowly with 1 per cent acetic acid The latter solution, clarified by filtration, was then noted in many instances to develop red fluorescence on exposure to light. (Porphobilinogen itself is not converted to porphyrin in this way) The porphyrin thus formed was removed by calcium phosphate precipitation and the resulting solution, although containing porphobilinogen, usually failed to exhibit porphyrin on heating at 80 C for 20 to 30 minutes (64)

This observation, together with demonstration of the Ehrlich-negative chromogen just referred to, led to the incorrect assumption that porphobilinogen itself is not converted to porphyrin (64) As a result of Westall's isolation and subsequent findings, it is now quite clear that this concept was wrong and we have abandoned it (161).

zene-petroleum ether, the melting point again is not sharp but somewhat lower, at 160 to 180 C. Thus far, even after extensive purification by chromatography and recrystallization, we have not observed the lower melting points (135 C.; 148 C.) with remelting at 170 C., so characteristic of coproporphyrin III methyl ester. Nevertheless, with the Chu-Green method (15a) it behaves entirely as coproporphyrin III, as also with the fluorescence quenching technic (120a). It is mainly ether-soluble, like coproporphyrin III, and unlike the type I isomer. The behavior of the decarboxylation mixture differs from that of any of the Waldenstrom porphyrins that we have observed, as isolated from acute porphyria urine. Whereas the total coproporphyrin mixture obtained from the Waldenstrom porphyrin in our hands usually behaves like a mixture of 75 per cent type I and 25 per cent type III and, less often, like a 50.50 mixture, that from the porphobilinogen uroporphyrin has characteristics of a mixture with at most 10 per cent isomer. This raises the question whether heating porphobilinogen *in vitro* is comparable to the *in vivo* process of porphyrin formation or to that in which porphobilinogen-containing urine is heated.

Cookson and Rimington (17a) now believe that at an alkaline pH, conversion is mainly to uroporphyrin I; at an acid pH, to uroporphyrin III. They also point out, on theoretical grounds, that at least *in vitro*, the condensation of porphobilinogen to porphyrin would be expected to yield significant amounts of isomers II and IV, as well as I and III. In this connection they recognize that Falk-Benson and Chu-Green chromatographic technics for separation of the type I and III isomers of uro- and coproporphyrin, respectively, do not permit separation of I and II, or III and IV. Thus the possibility must still be reckoned with that small amounts of II and/or IV may occur in nature, though they have not been identified. Cookson and Rimington (17a) recognized that *in vivo* formation need not be the same as that resulting from *in vitro* transformations, and indeed the great preponderance of type III isomer formation for heme synthesis clearly supports Rimington's earlier concept of a directed catalysis (105a).

Cookson and Rimington (17, 17a), Kennard (70), and Granick and Bogorad (53a) have shown that porphobilinogen is a monopyrrolic substance rather than a dipyrrylmethene as initially proposed by Waldenstrom and Vahlquist (144) The empiric formula of the crystals is $C_{10}H_{14}O_4N_2$ (17a) It is seen that the molecular weight of this compound is 226. Kennard (70) calculated a weight of 221 from

Nevertheless, the observation itself has been repeatedly confirmed and some interesting data have now been obtained relating to the basis for the difference, in respect to porphyrin formation, between pure porphobilinogen and this type of porphobilinogen solution. Due consideration was given to the reports of Westall (163), Herbert (65), and Brockman and Gray (9) that porphyrin formation from porphobilinogen is inhibited in solutions having relatively great ionic strength and/or buffer capacity, even when heated at pH 4, according to Waldenstrom's method Westall, nevertheless, found greater porphyrin formation at 0 5 N hydrochloric acid than at lower concentrations, and significant formation occurred in spite of a high buffer content We have repeatedly confirmed these observations Furthermore, it has been found that if the crude 1 per cent acetic acid (type A) solution of porphobilinogen (from aluminum oxide and following irradiation) is treated with mercuric acetate, as in the first step of the Westall method, and the precipitated porphobilinogen then redissolved in water after H_2S treatment, the aqueous (type B) solution, when heated, exhibits plentiful porphyrin formation, whereas prior to the mercury treatment none was demonstrable (161). This in itself could well be construed as due to elimination of interfering ions or buffer compounds It is believed, however, that some other explanation is necessary, as it has now been shown that porphyrin formation proceeds in the type A solution, without mercury precipitation, if sufficient hydrochloric acid is added. A critical difference has been noted at a pH of about 0 8 (161). Above this point, little or no porphyrin is formed, although considerable amounts are noted between 0 75 and 0 5. In the mercury-treated, or type B, solution considerable porphyrin is formed at pH 3 5 to 4 0. The fact that so much hydrochloric acid is required for porphyrin formation in the A solutions suggests that the porphobilinogen which they contain is of different form This does not necessarily imply any molecular change, indeed, the behavior, suggests more a complex with some urinary constituent, although evidence has been obtained (121) that there are different porphobilinogens This will be described by Dr. Schwartz in more detail elsewhere

Zinc has been considered a constituent of the possible complex, for it is complexly bound in the uroporphyrin excreted in the urine (150). However, it was found not to inhibit porphyrin formation after it had been suitably brought together with crystalline porphobilinogen in solution (160). Other common constituents of the urine have been

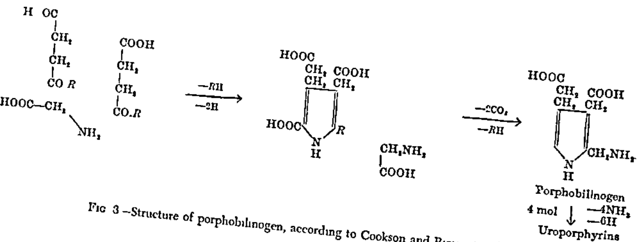


FIG 3 –Structure of porphobilinogen, according to Cookson and Rimington (17).

mainly type III, according to Falk-Benson and Chu-Green methods. Evidence was obtained that the uroporphyrin was a direct precursor of protoporphyrin, but that coproporphyrin was formed independently and was not intermediary. Bogorad and Granick (7a), using a Chlorella mutant which produces protoporphyrin, also found that porphobilinogen was converted to uro-, copro-, and protoporphyrin, as well as smaller amounts of less well-defined porphyrins. Of much significance was the observation that at 4 hours of incubation, uroporphyrin III was preponderant or almost solely represented, but that with further incubation this isomer disappeared and uroporphyrin I increased in amount, together with protoporphyrin (III). It was also found that the cell-free supernate of this mutant converted porphobilinogen to porphyrin in the same fashion. At room temperature, uro-III was first formed, later uro- I, but if the supernate was first heated at 55 C, only uro- I appeared. These interesting results again depended on differentiation of the isomers by the Falk-Benson method, and, as already seen, this fails to distinguish certain associations of I and III from III alone.

While there is now agreement that porphobilinogen is the monopyrrolic precursor of uro-, copro-, and protoporphyrin, differing results have been noted as to the possible intermediary role of uro- and coproporphyrin Alternate pathways, according to various workers, are shown in Figure 4 While this refers principally to the type III isomer series, the same possibilities are tenable for type I porphyrins, although no evidence of type I protoporphyrin formation has been reported Thus, for the present at least, uro- and coproporphyrin I must be regarded as side products of unknown function, if any. As seen in Figure 4, the recent study of Falk *et al* favors an independent synthesis of coproporphyrin There is, however, much indirect evidence suggesting that coproporphyrin III is intermediary (153), and negative results of *in vitro* experiments, even with living systems, scarcely exclude this possibility. The alternate possibilities shown in Figure 4 might explain the character of porphyrin excretion in the normal individual as well as in porphyria With regard to acute porphyria, a further conversion of much of the uroporphyrin III to protoporphyrin might account for the preponderance of uro- I in the Waldenström porphyrin, especially as isolated from unheated urine At the same time, the middle of the three alternate pathways in Figure 4, that proposed by Falk and co-workers, would make it easier to understand a simultaneous excess in formation and excretion of coproporphyrin

studied in this respect (160). Brockman and Gray had noted that their crude porphobilinogen solutions were relatively rich in uric acid; in fact, an absorption at 286 to 288 mμ, first thought related to porphobilinogen, was later recognized as being due to uric acid. Our type A solutions, failing to yield porphyrin on heating at pH 4.0, were also found to contain significant amounts of uric acid. Under suitably controlled circumstances, porphyrin formation was significantly inhibited when solutions of crystalline porphobilinogen containing uric acid were heated. No such effect was observed with creatinine, urea, or sodium chloride in similar amounts (160). While porphyrin formation was not completely prevented, as in the solutions described earlier, it was recognized that the conditions of the experiment were probably

TABLE 7.—EFFECT OF FRESH, NORMAL URINE ON CONVERSION OF PORPHOBILINOGEN* TO PORPHYRIN BY HEAT (80 C. FOR 30 MINUTES)

		Porphyrin, μg./Cc.	pH
a	1 cc. + 4 cc. urine + 0.2 cc. 3 N HCl	5.3	1.68
b	1 cc. + 4 cc. " + 0.2 cc. glacial acetic acid	15.0	3.62
c	1 cc. + 4 cc. " + 0.2 cc. 10% NaOH	2.7	9.60
d	1 cc. + 4 cc. H_2O + 0.2 cc. 3N HCl	105.6	1.02
e	1 cc. + 4 cc. " + 0.2 cc. glacial acetic acid	42.9	3.05
f	1 cc. + 4 cc. " + 0.2 cc. 10% NaOH	17.0	12.1
g	4 cc. urine + 1 cc. H_2O + 0.2 cc. 3N HCl	0.09	1.57

* 0.1 M NH_4OH solution crystalline porphobilinogen containing approx. 0.4 mg./cc.

not comparable to those under which uric acid might form a complex with porphobilinogen in the urine, or in the body. The result at least suggests that a complex of some type, possibly with uric acid, is the basis of our previous misconception (64). If such a complex exists in the native acute porphyria urine, the conditions must be such that it is often resolved on heating at pH 4.0, as this is frequently, but not always, associated with porphyrin formation. In this connection, the strongly inhibiting effect of normal urine on porphyrin formation from porphobilinogen has been noteworthy. Typical data are given in Table 7.

As already noted, the exact nature of the uroporphyrin formed on heating porphobilinogen *in vitro* is not yet clear, nor is the *in vivo* mechanism necessarily the same. Some light has been thrown on this question by studies with *in vivo* systems. Falk *et al.* (29a) find that porphobilinogen incubated with red cell hemolysate gives rise to uro-, copro-, and protoporphyrin. The uro- and coproporphyrins were

feces. It is reasonable to assume that in the latter condition there is a specific enzymic disturbance, in accord with the concept proposed years ago by Rimington (105a), a metabolic error which greatly enhances formation of type I isomers in the developing red cells of the bone marrow. The relative paucity of coproporphyrin I in hepatic porphyria may represent simply an expression of a lesser activity in this direction (*see* Fig. 4) on the part of the liver cell, as contrasted with the normoblast in erythropoietic porphyria. The pathway of conversion of porphobilinogen to the uroporphyrins is unknown. Granick (52b) has postulated a plan depending upon an intermediary tripyrrylmethene, somewhat according to the theory of Turner (132) and the syntheses of Corwin and Andrews (17b) Obviously there are many possibilities for an experimental approach to this problem

Several studies have been reported in which N^{15} glycine was given to individuals with porphyria in an attempt to throw light on the question of the interrelationship of the various porphyrins excreted Grinstein and co-workers (58, 58a) reported work from this laboratory in a case of erythropoietic porphyria N^{15} glycine was administered in two separate periods according to the technic of Shemin and Rittenberg (122). The results failed to establish that the uroporphyrin I was the immediate precursor of the coproporphyrin I, or vice versa (Fig 5). Similar results were reported by Gray and Neuberger (54) and London *et al* (77) Lowry *et al.* (79) later used N^{15} glycine to observe a case best classified as the mixed type of hepatic porphyria in remission. The feces contained relatively large amounts of proto- and coproporphyrin of the III series. It was of special interest that the peak concentration of N^{15} in the fecal protoporphyrin, after administering the glycine, was several days later than that in the coproporphyrin (Fig. 6) This was believed to have fundamental significance, but it could not be explained. This, too, may possibly be clarified by studies of the hepatic proto- and coproporphyrin in relation to catalase metabolism in experimental porphyria.

N^{15}-tagged uroporphyrin I, isolated during the study illustrated in Figure 5, was administered intravenously in a typical case of acute porphyria (154). The Waldenstrom porphyrin subsequently obtained from the urine in this case was significantly labeled with N^{15}. This only confirmed the belief (57, 150) that the Waldenstrom porphyrin includes uroporphyrin I, but because of the unknown dilution factor did not provide information about the relative proportions of series I and III being formed.

III. The direct transition of porphobilinogen to protoporphyrin without intermediary porphyrins such as uro- and copro (upper pathway, Fig. 4), would require either a preliminary alteration in the porphobilinogen molecule itself or the assumption that two or more different porphobilinogens are formed primarily. Schwartz's studies in this laboratory, to be described elsewhere, strongly support such a concept, and fail to confirm that of a transition of uro- to protoporphyrin An in-

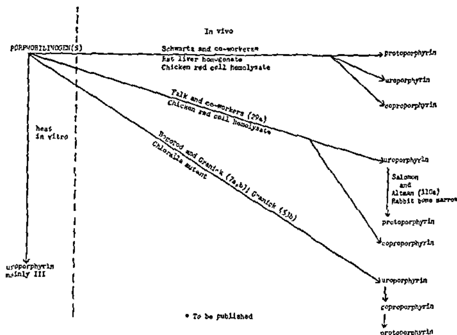


FIG 4 —Possible alternate pathways of porphyrin formation from porphobilinogen, as indicated by recent studies

dependent formation of coproporphyrin III and uroporphyrin I would just as readily explain the characteristic excesses in urine and feces in acute porphyria Quite possibly, both mechanisms obtain. The same is true for 7-, 6-, and 5-COOH type III porphyrins. The Waldenstrom porphyrin would then result from the molecular association of one or more of these (mainly 7-COOH according to our own studies) with uroporphyrin I.

In hepatic porphyria (including both acute and cutanea tarda types), relatively little coproporphyrin I is excreted, while in erythropoietic porphyria very large amounts are found, particularly in the

Brief additional consideration may be given to the Ehrlich-negative chromogen, already referred to It was stated (154, 155) that this was extracted from urine at Congo-negative reaction (pH 4 0–5 0). Subsequently, however, it was noted that extraction is more efficient at pH 3.2 (Congo gray blue), as is also the case with the Waldenstrom

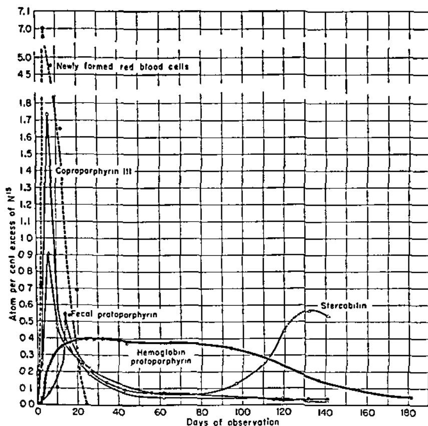


FIG 6 –Hemoglobin and porphyrin metabolism in chronic (mixed hepatic) porphyria, studied with the aid of N^{15} glycine (79)

porphyrin itself This is important, for although Herbert (65) has confirmed the finding of this chromogen, Brockman and Gray (9) state that they failed to observe it in the urines from 4 cases of acute porphyria. Nevertheless, as already mentioned, this chromogen can also be demonstrated by radiation of the 1 per cent acetic acid eluate from

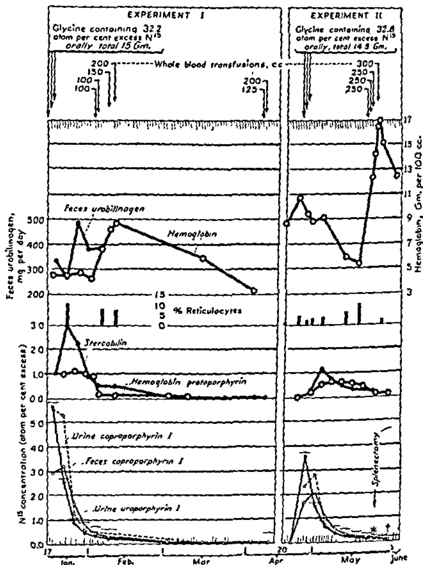


FIG 5 – Hemoglobin and porphyrin metabolism in erythropoietic porphyria, studied with the aid of N^{15} glycine (7a)

it is significantly positive. The reaction (149) is of great value in screening other members of a family for asymptomatic porphyria (67, 91, 142, 156). In our experience, the majority of individuals with porphyria have either siblings or other close relatives in whom tests give positive results

The belief that porphobilinogen or a closely related compound, perhaps an immediate derivative, is fundamentally related to the abdominal and nervous symptoms of the acute disease seems justified. Waldenstrom and Vahlquist (146) believed that uroporphyrin itself was not formed in the body except in trace amounts in the urinary bladder They based this belief on the fact that in their study of fresh urine and blood serum they were never able to detect porphyrin in the latter in acute cases. As already noted, Cookson and Rimington now regard the Waldenstrom porphyrin as a chemical artefact. While it is clear that porphobilinogen has a central role in the manifestations of acute porphyria, we have often observed significant amounts of preformed porphyrin in fresh urine, and since porphyrin is at times demonstrable in the circulating blood, cerebrospinal fluid, or fresh liver, it is doubtful that the Waldenstrom porphyrin can be regarded as wholly artefactual Its presence in the feces is also important, as porphobilinogen has not been demonstrated in bile or feces, in human porphyria While it is reasonable to assume that one of the essential interferences in acute porphyria involves the enzymatic conversion of porphobilinogen to porphyrin, this need not be regarded as total During relapse, the symptoms are regularly associated with the presence of large amounts of porphobilinogen in the urine On the other hand, it is usually lacking entirely in those cases of porphyria which are characterized solely by photosensitivity. Furthermore, in the latter, especially the erythropoietic group, porphyrinemia and porphyrinuria are characteristically much more marked, yet nervous or abdominal manifestations are completely absent.

The question might be raised whether the quantitative determination of uroporphyrin should be carried out before or after heating the urine. Since the marked increase which commonly occurs is both artefactual and variable, it is believed more reasonable to determine the native uro-type porphyrin and the porphobilinogen concentration separately, in 24 hour samples that have been kept alkaline (5 Gm Na_2CO_3) from the onset of the collection The porphobilinogen is relatively stable under these conditions

It would be important to know to what extent the chloroform-in-

aluminum oxide. The uroporphyrin formed in this way, or from ethyl acetate extracts, has been isolated repeatedly. As a rule, it has been a *type A Waldenstrom complex, but on occasion the 208 porphyrin* (type III) alone has been obtained. It may be noted that when porphyrin formation occurs on radiation of the 1 per cent acetic acid eluate, it is not at the expense of the porphobilinogen present, for the concentration does not diminish, and crystalline porphobilinogen itself, suitably dissolved, *is not converted to porphyrin by similar radiation* The Ehrlich-negative chromogen yields a small amount of porphyrin on very brief heating at 80 C., but the amount steadily diminishes after 5 minutes, in contrast to what is found on heating porphobilinogen solutions, in which the amount often continues to increase after 20 minutes and in some instances is still increasing at 45 minutes. The behavior of this Ehrlich-negative chromogen is reminiscent in many respects of that of uroporphyrinogen, the leukocompound of uroporphyrin obtained by brief reduction with sodium amalgam. The behavior in aqueous solution is entirely similar in respect to conversion to porphyrin on radiation. However, while uroporphyrinogen is partially extracted by ethyl acetate at pH 3 2 (HCl), it is quickly converted to porphyrin on shaking with 1 5 N hydrochloric acid, and it has thus far been impossible to separate chromogen from porphyrin in the manner described for the chromogen in the urine. It is worthy of note that the naturally occurring chromogen and the porphyrinogen obtained by amalgam reduction are also similar in respect to their conversion to porphyrin by iodine This is readily demonstrated by shaking an ethyl acetate extract with a dilute aqueous solution of iodine in 3 per cent aqueous sodium acetate solution which extracts the uro-type porphyrin as it is formed Iodine, however, does not *convert porphobilinogen to porphyrin, and indeed Granick and Bogorad's* (52a) recent study has shown that it combines with porphobilinogen, molecule for molecule, constituting strong evidence of its monopyrrolic nature

In acute porphyria, fresh urine usually contains porphobilinogen in readily demonstrable quantities Indeed, during relapse, the presence of porphobilinogen is a constant feature, as was emphasized years ago by Waldenstrom and Vahlquist (142, 146). During remission, the porphobilinogenuria persists in most cases, although in reduced degree (Table 4). The Ehrlich reaction of the urine becomes negative in some instances A negative porphobilinogen reaction during remission does not exclude the diagnosis of porphyria; as a rule, however,

I and 25 per cent type III isomers. In the case studied recently by Macgregor *et al.* (82), the uroporphyrin ester which was isolated melted at 259 to 261 C., and on decarboxylation yielded both copro-I and III. They state, that "the initial melting point of this mixture was 207–220, hence a preponderance of copro- I; the parent uroporphyrin was thus of the Waldenstrom ester type containing both isomers."

It is reasonable to believe that a larger proportion of preformed uroporphyrin I in the body, as indicated in Schmid's studies (115), may be responsible for the photosensitivity in this form of the disease. In the combined form, the appearance of abdominal or nervous manifestations has usually, but not invariably, been associated with the appearance of porphobilinogen in the urine. In 2 of our cases of the cutanea tarda type, however, mild abdominal pain was noted without demonstrable porphobilinogenuria Since concentration tests were not carried out, it is possible that small amounts were excreted and that larger or at least significant amounts were present in the body. The possibility that other abnormal pyrrol compounds are formed which are fundamentally related to these symptoms must also be considered.

Rimington and co-workers (82, 108) regard the cutanea tarda and combined types as a single, entirely independent form, quite unrelated to acute porphyria, regardless of whether the late cutaneous manifestations are associated in any given case with acute abdominal or nervous symptoms. Thus, in a case recently described by Macgregor, Nicholas, and Rimington (82), a boy of 14 had photocutaneous lesions together with evidence of liver injury, jaundice, abdominal pain, and nervous system involvement, a combination of abnormalities which terminated fatally after several well-defined attacks Porphobilinogen was observed in the urine only during the final hospital admission, shortly before death. According to our classification, this case would be categorized as one of the mixed type of hepatic porphyria Rimington, however, regards this type of case as fundamentally distinct from acute porphyria and not representative of a mixture with, or transition from the cutanea tarda type. He places considerable emphasis on differences he has observed in respect to the amount of porphyrin in the feces of such cases during remission In the instance just referred to, the feces–urine porphyrin ratio was high during remission and relatively low during relapse. In a considerable number of fecal porphyrin determinations we have found no sharp distinction between the two forms insofar as uroporphyrin is concerned The coproporphyrin excretion, it is true, is usually greater in the cutanea

soluble Ehrlich aldehyde test for porphobilinogen (149) or the finding of excessive uroporphyrin in the urine is pathognomonic of porphyria. Hammond and Welcker (63) examined 1,000 random urine samples from as many individuals in a general hospital population and failed to find a single one that was positive for porphobilinogen. This at least points to a relatively high degree of specificity. No comparable data with respect to uroporphyrin have been reported. The latter problem is rendered more difficult by the occurrence of very small amounts under normal circumstances. No body of data on the normal range has been reported, and this information is much needed. We have repeatedly observed from 10 to 20 μg. in 24 hour urine samples from individuals who were either normal or without serious disease, but the normal range may well exceed this. In one group of cases (161), both porphobilinogen and excessive uroporphyrin have been encountered in association with a variety of diseases, such as cirrhosis of the liver, carcinomatosis, Hodgkin's disease, and nervous system affections, including poliomyelitis. The significance of these observations has not been determined. The interesting question, of course, is whether these are individuals with a latent trait, in other words, whether the appearance of porphobilinogen and excessive uroporphyrin necessarily indicates a constitutional abnormality or inborn error of metabolism or whether it may simply represent a disturbance of purely acquired type. Thus far at least, it appears that most patients with cirrhosis and hepatic disease, malignancy, and nervous affections do not customarily exhibit such increases, which favors the concept that in these exceptional individuals there is an abnormal trait. Nevertheless, this question deserves further study. Bénard *et al.* (5) believe that such instances represent latent porphyria and that there are certain individuals with smaller amounts of porphyrin in the urine representing milder examples of the disease.

In the cutanea tarda and combined types of hepatic porphyria, the urine has been found to contain a Waldenström uroporphyrin complex the methyl ester of which has a slightly higher mean melting point than that isolated in acute porphyria (157). Thus, in 8 isolations, the range was from 259 to 276, with a mean of 266.2, while in 31 isolations from intermittent acute cases the range was 253 to 274, with a mean of 259.1 This difference was significant and suggested the presence of a slightly greater proportion of uroporphyrin I in the complex. On the basis of methods referred to in the foregoing, Nicholas and Rimington (95) conclude that the uroporphyrin from cutanea tarda porphyria cases is composed of about 75 per cent type

tarda cases. Representative data* are shown in Table 8 In none of these cases were there any symptoms—abdominal, nervous, or cutaneous—at the time of the determination

Quite recently urine and fecal porphyrins were studied during a complete remission of symptoms in a case of combined type. This individual,† a man of 25, previously had both photocutaneous lesions and abdominal and nervous manifestations. The disease was familial, some individuals having only the intermittent acute form In this case, the 24 hour urine (unheated) contained 76 units‡ of porphobilinogen, 4,643 µg. of uroporphyrin, and 436 µg. of coproporphyrin, the feces contained 1,232 µg of coproporphyrin and 689 µg of uroporphyrin over a 4 day period. Thus it is evident that even in remission, and despite the history of "cutanea tarda" manifestations, the total urinary porphyrin may exceed that in the feces

It may be re-emphasized that in acute porphyria and in some of the combined cases, as in the foregoing instance, a large fraction of the output of abnormal pyrrol compounds is represented by porphobilinogen, which is excreted only in the urine, and that porphobilinogen is completely lacking from the urine in the simple cutanea tarda cases It is evident that this constitutes an important difference, but it seems inadequate to establish the two forms as separate and unrelated entities in the face of transitional cases and of families in which both forms are represented. Assuming that the two forms are fundamentally related, the factor or factors which determine whether porphobilinogen or preformed porphyrin formation is dominant in the liver at any given time remain to be elucidated Nothing as yet is known about the fundamental basis of the error responsible for this excessive formation Studies of experimental porphyria may open the way to clarification of this problem.

TREATMENT

Porphyria Erythropoietica

As already indicated, splenectomy is of definite value and may even be lifesaving in certain cases Although experience is thus far

* Unpublished study carried out with Violet Hawkinson, Irene Bossenmaier, and Mary Ann Farisy

† Studied *through the courtesy of the Army Medical Service Graduate School* and Walter Reed Hospital, Washington, D C

‡ One unit approximately equals 1 mg (161)

TABLE 8.—PORPHYRIN DATA FROM INTERMITTENT ACUTE AND CUTANEA TARDA FORMS OF PORPHYRIA IN REMISSION

Case	Type*	Fecal Porphyrins µg			Urinary Porphyrins, µg		
		Uro-	Copro-	Proto-	Uro-	Copro-	Porphobilinogen
E M	C T	167/100 Gm	8,112/100 Gm	1,200/100 Gm.	5,928/day	498/day	neg.
M B	I A	neg	680/100 Gm.	2,380/100 Gm.	53/100 cc.	18/100 cc.	+
E H	C T	240/100 Gm	8,400/100 Gm	840/100 Gm.	14,700/day	1,670/day	neg
B R	I A	500/100 Gm	300/100 Gm	—			
H K	I A	1,104/100 Gm	1,014/100 Gm	1,504/100 Gm.	820/day	368/day	38 u./day
R T	I A	1,103/day	2,083/day	—	6,450/day	540/day	71 u./day
		trace	4,558/day				
C B	C T	trace	2,638/100 Gm	—	1,680/day	196/day	neg.
		61/day	809/day		800/day	228/day	neg.
M J	I A.	720/100 Gm	2,424/100 Gm	1,320/100 Gm.	23,700/day	750/day	55 u./day
		2,210/100 Gm	2,380/100 Gm				
C K	I A	67/day	1,408/day	—	35,500/day	463/day	76 u./day
M C	C T	7,600/100 Gm.	10,396/100 Gm	—	16,380/day	298/day	neg.
		1,312/100 Gm	2,195/100 Gm				
H C	C T	1,329/day	9,032/day				
I C	C T	1,320/day	2,970/day		3,562/day	544/day	neg.
O S	I A	838/100 Gm	676/100 Gm	1,600/100 Gm	329/100 cc.	18.5/100 cc.	1.4 u./100 cc.

*C T cutanea tarda I A intermittent acute

tarda cases. Representative data* are shown in Table 8. In none of these cases were there any symptoms—abdominal, nervous, or cutaneous—at the time of the determination

Quite recently urine and fecal porphyrins were studied during a complete remission of symptoms in a case of combined type. This individual,† a man of 25, previously had both photocutaneous lesions and abdominal and nervous manifestations The disease was familial, some individuals having only the intermittent acute form In this case, the 24 hour urine (unheated) contained 76 units‡ of porphobilinogen, 4,643 μg. of uroporphyrin, and 436 μg. of coproporphyrin, the feces contained 1,232 μg of coproporphyrin and 689 μg of uroporphyrin over a 4 day period. Thus it is evident that even in remission, and despite the history of "cutanea tarda" manifestations, the total urinary porphyrin may exceed that in the feces

It may be re-emphasized that in acute porphyria and in some of the combined cases, as in the foregoing instance, a large fraction of the output of abnormal pyrrol compounds is represented by porphobilinogen, which is excreted only in the urine, and that porphobilinogen is completely lacking from the urine in the simple cutanea tarda cases It is evident that this constitutes an important difference, but it seems inadequate to establish the two forms as separate and unrelated entities in the face of transitional cases and of families in which both forms are represented Assuming that the two forms are fundamentally related, the factor or factors which determine whether porphobilinogen or preformed porphyrin formation is dominant in the liver at any given time remain to be elucidated. Nothing as yet is known about the fundamental basis of the error responsible for this excessive formation Studies of experimental porphyria may open the way to clarification of this problem

TREATMENT

Porphyria Erythropoietica

As already indicated, splenectomy is of definite value and may even be lifesaving in certain cases Although experience is thus far

* Unpublished study carried out with Violet Hawkinson, Irene Bossenmaier, and Mary Ann Farisy

† [illegible] Medical Service Graduate School and [illegible]

‡ [illegible]

quite limited, it appears that splenectomy is indicated in any case of this type in which definite evidence of increased hemolytic activity is found (increased fecal urobilinogen excretion and increased reticulocyte percentage). The first recorded instance was that of de Marval and Pons (84a). The hemolytic anemia was apparently ameliorated, but it is not clear whether the photosensitivity was improved. Both of our cases (2, 115) were greatly benefited, as regards both photosensitivity and the hemolytic activity. The second of the 2 patients, however, has had a recurrence of bullae on the fingers, 2 years after splenectomy. These seem to be related to trauma at least as much as to light. Otherwise, she is well. In Zuelzer and Kaplan's (162) case, a 2 year remission of the hemolytic anemia occurred, followed, however, by a partial relapse; further details are not available. In Gray and Neuberger's (54, 55) case, splenectomy was not beneficial. Since this patient was much older (a 32 year old man) as compared to the others, all of whom were very young girls, it will be important in the future to determine whether age and sex are of significance with respect to the effect of splenectomy.

In the second of our cases, riboflavin, in 10 mg. amounts intravenously for 8 days and 30 mg. orally for an additional 23 days, was without effect on the magnitude of porphyrin excretion. In the same case during another period, ACTH, 10 mg. every 8 hours for 10 days, was also without demonstrable effect.

Nothing new has been advanced with regard to protection from sunlight, but further studies are needed on the value of certain newer materials for sun protection. Developed during the war, they are now on the market, and they might have genuine merit in this form of the disease.

Intermittent Acute Porphyria

Changes in this disease for better or worse are characteristically so abrupt and unpredictable and the variations in pattern and severity a [illegible] difficult F [illegible] e stand- p [illegible] ne, that the time is often too short to provide a suitable base line for the trial of any given substance Nevertheless, some important information has been gained in recent years which permits a somewhat more optimistic attitude, although this form of the disease must still be regarded

as unusually treacherous and often lethal, principally because of bulbar or respiratory paralysis, or coma. Treatment is directed toward aborting or ameliorating the attack and to the symptomatic relief of pain.

There is some inconclusive evidence that ACTH or cortisone may be of benefit in the earlier stages of an acute relapse. The author is now aware of the outcome in 15 patients having acute attacks. Prompt and often dramatic improvement occurred in 8 of them, and in 4 of these, the attack was actually completely aborted within 36 to 48 hours after the start of ACTH therapy. These data at least suggest that if the duration of the relapse has not been too long and nervous system and visceral changes are not irreversible, ACTH or cortisone therapy is probably not harmful and may be of value. Patients in whom no response was observed were for the most part those with relapses of longer duration This was also true in the case reported by Oltman and Friedman (96). The photograph of their patient reveals quite well the chronic debility and incapacitation which may occur in "acute" porphyria The appearance of the hands and feet is [illegible]

failure of ACTH in a patient having abdominal and nervous system symptoms for 4 weeks. Goldberg *et al.* (53) found no significant lowering of uroporphyrin excretion in 1 patient receiving ACTH for 1 week. Very recently, Olson and Stiles (95a) observed 2 cases terminating fatally in which there was some reason to believe that cortisone may have had an adverse effect This is difficult to judge, because of the rapidity with which improvement or death supervenes in these cases, without cortisone. Nevertheless, cortisone or ACTH may very well be either beneficial or harmful in effect, depending upon unknown factors in individual cases. It is fully recognized that coincidence has not been excluded in the cases in which prompt improvement has occurred It seems not unlikely, however, that in these instances an abrupt, nonspecific change of metabolism by the ACTH was responsible for inducing the remission. The impression is very strong that in this disease, the borderline between remission or latency and relapse is very easily shifted, although altogether too little is known of the factors responsible for the change in either direction. Further study of this question is needed It is believed, however, that in view of the possibility of adverse effect in certain cases ACTH or

quite limited, it appears that splenectomy is indicated in any case of this type in which definite evidence of increased hemolytic activity is found (increased fecal urobilinogen excretion and increased reticulocyte percentage). The first recorded instance was that of de Marval and Pons (81a). The hemolytic anemia was apparently ameliorated, but it is not clear whether the photosensitivity was improved. Both of our cases (2, 115) were greatly benefited, as regards both photosensitivity and the hemolytic activity. The second of the 2 patients, however, has had a recurrence of bullae on the fingers, 2 years after splenectomy. These seem to be related to trauma at least as much as to light. Otherwise, she is well. In Zuelzer and Kaplan's (162) case, a 2 year remission of the hemolytic anemia occurred, followed, however, by a partial relapse; further details are not available. In Gray and Neuberger's (54, 55) case, splenectomy was not beneficial. Since this patient was much older (a 32 year old man) as compared to the others, all of whom were very young girls, it will be important in the future to determine whether age and sex are of significance with respect to the effect of splenectomy

In the second of our cases, riboflavin, in 10 mg. amounts intravenously for 8 days and 30 mg. orally for an additional 23 days, was without effect on the magnitude of porphyrin excretion In the same case during another period, ACTH, 10 mg. every 8 hours for 10 days, was also without demonstrable effect.

Nothing new has been advanced with regard to protection from sunlight, but further studies are needed on the value of certain newer materials for sun protection Developed during the war, they are now on the market, and they might have genuine merit in this form of the disease.

Intermittent Acute Porphyria

Changes in this disease for better or worse are characteristically so abrupt and unpredictable and the variations in pattern and severity are so great that any controlled study of therapy is unusually difficult Furthermore, the need of alleviation is often so urgent, from the standpoint either of pain or possible avoidance of a fatal outcome, that the time is often too short to provide a suitable base line for the trial of any given substance. Nevertheless, some important information has been gained in recent years which permits a somewhat more optimistic attitude, although this form of the disease must still be regarded

Cutanea Tarda Type of Porphyria

Castex *et al* (15) reported striking benefit from folic acid in 2 cases of this form of the disease. As yet, no other report has confirmed this result. Our experience has been disappointing, no definite effect being noted, either on photosensitivity or on porphyrin excretion In 2 of our patients, crystalline vitamin B_{12}, and in 1, riboflavin administration, have given seemingly genuine improvement, at least very gratifying to the patient. Whether this is coincidental or not remains to be seen. Tappeiner (129) has reported on the use of vitamin B_{12} and B complex in 12 cases of pure cutanea tarda type Symptomatic benefit was observed, but no effect on uroporphyrin excretion was noted. Questionable reduction of urinary coproporphyrin excretion was described. Further trials with these substances are needed.

EXPERIMENTAL PORPHYRIA

Stokvis (127) first described the experimental production of a marked increase in porphyrinuria in rabbits by the use of sulfonal. He believed that the porphyrin was derived from blood in the intestinal tract. In view of recent information, however, this seems very unlikely. It is impossible to determine the exact nature of the excessive porphyrin which Stokvis observed Years later, Waldenstrom and Wendt (145) carried out similar studies, but found no increase in uroporphyrin, although abnormal coproporphyrinuria was readily produced Somewhat earlier, however, Fischer and Duesberg (42) had reported finding an ether-insoluble or uro-type porphyrin in the urine of sulfonal-poisoned rabbits. But in none of these experiments was a condition produced which could properly be designated as porphyria, either on the basis of physiologic manifestations or the excretion of porphobilinogen or excessive uroporphyrin.

Studies of the past few years in this laboratory by Pimenta de Mello (97–99), Schmid, Schwartz, and associates (111–113, 116, 121, 159), have delineated two forms of experimental porphyria—erythropoietic and hepatic—corresponding in some respects to the human types, as described in the foregoing The first work leading to the development of the erythropoietic form in rabbits was that of Pimenta de Mello, in which it was shown that the photodynamic effect of rose bengal results in a marked but rather transitory increase of

cortisone should only be used briefly. Our own policy has been to continue it for not longer than 5 to 7 days. Experience thus far indicates that remission with ACTH therapy will occur within 4 days, if at all.

Folic acid has been reported to cause reduction in porphyrin excretion and amelioration of the attack (15, 73). This could not be confirmed in several cases of our own series. Stich (125, 126) noted a beneficial effect of riboflavin in all forms of porphyria, but although we have used riboflavin phosphate intravenously as well as riboflavin by mouth, in amounts ranging from 20 to 40 mg. daily, we have not seen any evidence of effect in the acute form of the disease. Nevertheless, Lups and van Dijk (80) have reported good results in 1 case receiving 12 mg daily. Further study of its effect is needed in the cutanea tarda type We have also failed to observe benefit or reduction of porphyrin excretion following large amounts of crystalline vitamin B_{12} given parenterally. This is of special interest in view of the fact that Frank *et al.* (49) have reported a marked reduction in coproporphyrin III excretion in lead poisoning as a result of vitamin B_{12} administration. This is reminiscent of the effect of B_{12} in certain experimental studies, as noted by Pimenta de Mello (97). Verlingstad (139) recommended prostigmine for abdominal pain. We have seen apparent benefit in 2 patients and none in several others. Wehrmacher (162) first suggested use of tetraethyl ammonium (Etamon) and observed striking relief in 1 patient, on many occasions. We, too, have repeatedly observed marked or even complete relief of abdominal pain with Etamon lasting for 4 to 12 hours. It is noteworthy that at least some patients with acute porphyria exhibit remarkable sensitivity to hexamethonium Thus, in 2 of our patients, 10 mg. intramuscularly was followed by a fall in the blood pressure to shock levels and norepinephrine was necessary to avert a catastrophe Because of the repeated beneficial effect of Etamon in 1 of our patients, a bilateral splanchnicectomy was recently carried out by Dr Lyle French (of the Division of Neurosurgery, University of Minnesota Hospital), with complete relief of pain Postoperatively this patient had moderate hypotension but stabilized well after the first 3 days and has felt very well since (10 months) Judging from this limited experience, this operation may prove to be the procedure of choice for patients with enduring or frequently recurring upper abdominal pain, especially when it is clearly relieved by Etamon It is, of course, too soon to tell whether the pain will recur at some time in the future.

porphyrinuria in animals. And last, sunlight has been reported to exacerbate the porphyrinuria of lead poisoning, at least under certain conditions. The excessive porphyrin content of the bone marrow normoblasts in the experimental erythropoietic form of the disease due to lead and phenylhydrazine is similar to that observed in the human erythropoietic type Fluorescence microscopy of other tissues in these animals has failed to indicate any significant extramedullary porphyrin formation.

More recently, Schmid and Schwartz (113) have discovered a *means of producing an experimental porphyria of hepatic type. This* is regularly brought about by continued administration of the semicarbamide Sedormid (allylisopropylacetyl-carbamide) in rabbits, rats (1a), and mice. The porphyrin metabolism of dogs is also affected in similar fashion, although much less markedly.

In initiating this work, Schmid was induced to try Sedormid because of a case report by Duesberg (25) in which typical clinical and laboratory findings of acute porphyria, ending with death, followed a long-continued ingestion of large amounts of Sedormid. While a causal relationship was not established in this case, it seemed desirable to test the effect of the substance in animals and this fortunately has provided a valuable tool in the further study of porphyrin metabolism and chemistry.

Up to the present time, the disease has been studied more extensively in rabbits. Administration of Sedormid continued for several days at a dosage level of 200 mg per kilogram per day results in a striking picture that can only be designated as a form of porphyria. "Clinically," the animal develops marked gastrointestinal changes, with areas of spasm and dilatation (113) It is most outspoken in relation to the stomach, which becomes enormously dilated (Fig 7) and eventually perforates, this being the usual cause of death Other areas of spasm and dilatation are also noted in the bowel, quite similar to that encountered in intermittent acute porphyria in man Many of the animals develop weakness or outspoken paralysis, especially of the hind legs. The uro-type porphyrins in the urine at times equal or exceed the amount excreted in a similar period by patients with acute porphyria The behavior of these porphyrins differs from the Waldenstrom porphyrin isolated from patients The bulk of the porphyrin in the rabbit urine is type III isomer, as shown by a clear preponderance of coproporphyrin III after decarboxylation. This in itself serves as a control observation with respect to the preponderance of type I

coproporphyrin III (97). He also observed that light exposure significantly increases the coproporphyrinuria of lead poisoning in rabbits (98). Schmid and co-workers (111, 112) subsequently demonstrated that lead itself causes a significant increase in the formation of uroporphyrin which is best demonstrated in the bone marrow. Interestingly enough, this was largely the type I isomer, while the excessive coproporphyrin both in the marrow and the urine was type III Schwartz *et al.* (121) then showed that if lead poisoning is combined with excessive hemolytic activity, as produced by phenylhydrazine, a much greater porphyrin formation and excretion is effected and a form of erythropoietic porphyria is produced. The further addition to these agents of the photodynamic effect of rose bengal causes still further increase, the urine becoming dark red. The animals survive only short periods of these combined insults, but with proper adjustment of dose they recover completely. Apart from the purely acquired nature of this disturbance, there are certain other important differences between it and the human erythropoietic porphyria. Thus, in the latter the coproporphyrin excreted is almost entirely type I isomer, while in the experimental disease it is mainly type III. Of even more interest is the fact that in the experimental disease porphobilinogen is regularly encountered in significant amount, although less than that observed in the experimental hepatic porphyria. Whereas in the latter form it appears likely that the porphobilinogen is formed in the liver, it has thus far been impossible to demonstrate it either in bone marrow or liver of the lead-phenylhydrazine-poisoned rabbits. Further studies of this question are in progress

Thus far it has not been possible to produce photocutaneous lesions in these animals, but the studies bearing on this point have been very preliminary in character and with proper adjustment of conditions, perhaps merely continued exposure to sunlight, this should be possible It must be borne in mind that even in the very chronic erythropoietic porphyria of man relatively brief artificial radiation of the skin with light of the proper wave length has usually not produced lesions It is conceivable that more extensive or prolonged exposure to sunlight is required Indeed, there is some reason to believe, both on the basis of human and animal observations, that such exposure will then bring about photocutaneous lesions As was mentioned earlier, in photosensitive porphyria of man, red urine may first be noted after exposure to sunlight in the spring or summer. Further, there is Pimenta de Mello's observation that photodynamic activity promotes

TABLE 9.—PORPHYRIN CONCENTRATIONS IN VARIOUS ORGANS AND EXCRETA OF RABBITS WITH EXPERIMENTAL (SEDORMID) PORPHYRIA (113)

Organ or Excreta	No of Rabbits	µg per 100 Gm or 100 Ml.					
		Uroporphyrin*		Coproporphyrin		Protoporphyrin	
		Range	Mean	Range	Mean	Range	Mean
Erythrocytes	5	0–1.8	0.9	tr – 6.6	1.9	10 – 12	32
Bone marrow	5	0–1.5	0.5	tr – 14	6.4	15 – 64	42
Spleen	4	0–13.9	5.8	tr. – 18	7.8	24 – 30	27
Liver, fresh	7	9–233	108	72 – 167	132	176 – 1,700	819
Liver, heated	7	19–1,042	425	65 – 224	139	133 – 1,820	585
Kidney	4	30–120	76	34 – 162	116	46 – 74	66
Brain	3	0–1.9	0.6	tr – 2.8	1.6	3.8–5.5	4.1
Plasma	4	0–tr	tr.	2.5–8.1	5.1	5.7–10.4	8
Bile	5	383–5,480	2,122	4,600 –39,600	19,800	13,800 –86,400	40,000
Feces	4	90–2,180	870	1,980 – 5,900	3,560	5,500 –12,800	9,100
Urine	7	13,800–76,130	24,750	260 – 780	490	0	0

* This includes all ether-insoluble uro-type porphyrins

which we find in the human material. The group of uro-type porphyrins in the rabbit urine includes a number of different components, as shown by the repeated finding of some 10 zones on column chromatography with calcium carbonate (117). The liver and bile of these animals are unusually rich in porphyrin content (Table 9). As noted, the increase of proto- and coproporphyrin definitely precede that of the uro-type porphyrins. The liver and bile contain large amounts of copro- and protoporphyrin and smaller amounts of porphobilinogen



FIG 7—Rabbit with experimental (Sedormid) porphyria, note greatly distended stomach, barium meal was introduced through a catheter (Courtesy of Dr R Schmid)

Unlike human porphyria, a minor fraction of the porphobilinogen is found in the bile, the majority either being converted to porphyrin or excreted in the urine. Very little uro-type porphyrin is found in the bile, which contains mainly copro- and protoporphyrin, conversely, no protoporphyrin is found in the urine, which contains large amounts of uro- and coproporphyrin. The porphyrin content of bone marrow and spleen is essentially normal, the kidneys are relatively rich in uro- and coproporphyrin, with some porphobilinogen, and very little protoporphyrin The liver, gallbladder, and bile ducts, viewed under Wood's light, exhibit a striking red fluorescence due to the great excess of copro- and protoporphyrin, especially in the bile.

It was logical to explore the possibility that a disturbed metab-

8 Borst, M, and Konigsdorffer, H, Jr: *Untersuchungen uber Porphyrie mit besonderer Berucksichtigung der Porphyria congenita* (Leipzig S Hirzel, 1929).
9 Brockman, P. E, and Gray, C. H Studies on porphobilinogen, Biochem J 54 22, 1953
10 Brunsting, L A, Mason, H L, and Aldrich, R A.: Adult form of chronic porphyria with cutaneous manifestations Report of seventeen additional cases, J.A M A 146,1207, 1951.
11 Calvy, G L · Porphyria. A consideration in surgical diagnosis, Surg, Gynec & Obst 90 716, 1950
12 Calvy, G L; Jarnszewski, E. J, and Carroll, H. H: Porphyria: Clinical observations and a family vignette, Ann Int Med 34.767, 1951.
13 Canivet, J, Fallot, P., and Poidatz, J · Étude des porphyrines urinaires de neuf cas de porphyrie aigue, Semaine hôp Paris 28 3883, 1952
14 Carrié, C *Die Porphyrine, ihr Nachweis, ihre Physiologie und Klinik* (Leipzig Georg Thieme, 1936)
15 Castex, M. R; Lopez Garcia, A, and Zelasco, J F · Dos casos de porfiria, cutanea y abdominal, tratados con ácido fólico, Prensa méd argent. 35 907, 1948
15a Chu, E J; Green, A. A, and Chu, T. C: The chromatography of methyl esters of porphyrins, J Biol Chem 190 643, 1951.
16 Church, W. A. Researches on turacine, an animal pigment containing copper, Proc Roy, Soc, London 17 436, 1869
17. Cookson, G H, and Rimington, C Porphobilinogen Chemical constitution, Nature, London 171.875, 1953
17a Cookson, G H, and Rimington, C. Porphobilinogen In press (Manuscript seen through courtesy of the authors.)
17b Corwin, A H, and Andrews, J S Studies in the pyrrole series II The mechanism of the aldehyde synthesis of dipyrrylmethenes, J Am Chem Soc 58 1086, 1936.
18 Davies, D Acute porphyria and associated electrolyte changes, Brit M. J 1 846, 1949
19 Denny-Brown, D, and Sciarra, D Changes in the nervous system in acute porphyria, Brain 68 1, 1945
20 Derrien, E, and Benoit, C. [Abstract of a study of the urines of a case of acute porphyria], Bull Soc chim de France s 4 43 522, 1928
21 Derrien, E, and Cristol, P Zincoporphyrinurie, Compt rend Soc biol 103 126, 1930
22 Dobriner, K Simultaneous excretion of coproporphyrin I and III in a case of chronic porphyria, Proc Soc Exper Biol & Med 35 175, 1936
23 Dobriner, K, and Rhoads C P The porphyrins in health and disease, Physiol Rev 20 416, 1940
24 Dorken, H Zur Klinik der akuten Porphyrie, Ztschr klin Med 50 260, 1953
25 Duesberg, R Toxische Porphyrie, Munchen med Wchnschr 79 1821, 1932.
26 Edmondson, P, and Schwartz, S Studies of the porphyrins An improved method for the decarboxylation of uroporphyrin, J. Biol Chem 205 605, 1953

olism of one of the heme-protein enzymes in the liver was the basis of the excessive amounts of porphyrin and porphobilinogen in these animals. Schmid and Schwartz (114) have now shown clearly that at the peak of the Sedormid effect the liver catalase activity is reduced to 5 to 10 per cent of normal. Preliminary studies indicate that this is due to an actual diminution in amount, not simply an inhibition of activity. The fact that such large amounts of protoporphyrin are found indicates that the fundamental disturbance is in the final fabrication of the catalase molecule, i e, the assembly of its protein moiety, its iron and protoporphyrin, but not in the construction of its protoporphyrin The basis of the great accumulation of copro- and uroporphyrin, and porphobilinogen is not yet clear. Possibly this is due to an overstriving or abnormal compensation, with the appearance or even new formation of compounds normally not in evidence or present in minimal amount.

Acknowledgment is made of support of certain of the above studies in the author's laboratory, under contracts with the Atomic Energy Commission, and the Surgeon General's Office, Preventive Medicine Division, U.S.A., the latter contract being sponsored by the Commission on Liver Disease of the Armed Forces Epidemiological Board

REFERENCES

1 Abrahams, A; Gavey, C J, and MacLagan, N F A fatal case of acute porphyria with unusual features, Brit M J 2 327, 1947
1a Aldrich, R A Personal communication
2 Aldrich, R A, et al Photosensitive or congenital porphyria with hemolytic anemia I Clinical and fundamental studies before and after splenectomy, Blood 6 685, 1951
3 Amatuzio, D S, Weber, L., and Nesbitt, S Bilirubin and protein in the cerebrospinal fluid of jaundiced patients with severe liver disease, with and without hepatic coma, J Lab & Clin Med 41 615, 1953
4 Anderson, T M Hydroa aestivale in two brothers, complicated with the presence of haematoporphyrin in the urine, Brit J Dermat 10 1, 1898
5 Bénard, H, et al Porphyrie à forme abdominale aigue avec signes biologiques frustes, Bull et mém Soc méd hôp Paris 68.143, 1952.
6 Blum, H F, and Hardgrave, L E Spectral region of photosensitivity in hydroa aestivale seu vacciniforme with porphyrinuria, Proc Soc Exper Biol & Med 34 613, 1936
7 Blum, H F, and Pace, N Studies of photosensitization by porphyrins, Brit J Dermat & Syph 49 465, 1937
7a Bogorad, L, and Granick, S Conversion of porphobilinogen to porphyrins, Abst Meeting Am Chem Soc Sept 1953, p 13c, no 34
7b Bogorad, L, and Granick, S The enzymatic synthesis of porphyrins from porphobilinogen, Proc Nat. Acad Sc., 39.1176, 1953.

45 Fischer, H, and Orth, H · *Die Chemie des Pyrrols* (Leipzig. Akademische Verlagsgesellschaft, 1937), Vol II, Part I.

46 Fischer, H, and Hofmann, H. J.: Über die Konstitution des Uro- und Muschelschalenporphyrins. Nachweis von Uroporphyrin III bei kongenitaler Porphyrie, Ztschr. physiol Chem 246.15, 1937.

47. Fischer, H, und Muller, A: Synthese einer Tetramethyl-porphin-tetraessigsaure Ein Beitrag zum Konstitutionsproblem des Uroporphyrins, Ztschr physiol Chem. 246 31, 1937.

48 Fraenkel, E · Experimentelles uber Hamatoporphyrie Virchows Arch path Anat 248 125, 1924.

49 Frank, O; Lachnit, V, and Neumayr, A: Influence of Vitamin B_{12} on lead porphyrinuria, Acta haemat. 8 42, 1952

50 Garcin, R, and Lapresle, J Manifestations nerveuses des porphyries, Semaine hôp Paris 26 3404, 1950

51 Garrod, A E *Inborn Errors of Metabolism* (2d ed; London. Henry Frowde, 1923)

52 Gibson, Q H, and Harrison, D C A note on the urinary uroporphyrin in acute porphyria, Biochem J. 46 154, 1950

53. Goldberg, A, Macdonald, A. C, and Rimington, C. Acute porphyria, experimental treatment with A C T H, Brit M J 2 1174, 1952.

53a Granick, S, and Bogorad, L. Porphobilinogen, a monopyrrole, J Am Chem Soc. 75 3610, 1953

53b. Granick, S. Personal communications, see also references

54 Gray, C H, and Neuberger, A Investigations on porphyrin formation in congenital porphyria with the aid of N^{15}, Biochem J 44 xiv, 1949

55 Gray, C H, and Neuberger, A · Studies in congenital porphyria· I Incorporation of N^{15} into coproporphyrin, uroporphyrin and hippuric acid, Biochem. J 47.81, 1950

56. Gray, C. H Acute porphyria, report of a case, Arch Int Med 85 459, 1950

57. Grinstein, M, Schwartz, S, and Watson, C J Studies of the uroporphyrins I The purification of uroporphyrin I and the nature of Waldenstrom's uroporphyrin, as isolated from porphyria material, J Biol Chem 157 323, 1945

58 Grinstein, M, *et al*. An isotopic study of porphyrin and hemoglobin metabolism in a case of porphyria, J. Biol. Chem 179 983, 1949

58a Grinstein, M, *et al*. Photosensitive or congenital porphyria with hemolytic anemia II Isotopic studies of porphyrin and hemoglobin metabolism, Blood 6 699, 1951.

59 Grotepass, W.· Zur Kenntniss des im Harn auftretenden Porphyrins bei Bleivergiftung, Ztschr. physiol Chem 205 193, 1932

60 Gunther, H · Die Hamatoporphyrie, Deutsches Arch klin Med. 105·89, 1911.

61 Gunther, H.. Die Bedeutung der Hamatoporphyrie in Physiologie und Pathologie, Ergebn allg Path u path Anat 20 607, 1922

62 Gunther, H.: Hamatoporphyrie, in Schittenhelm, A (ed) *Handbuch der Krankheiten des Blutes und der blutbildenden Organe* (Berlin. Julius Springer, 1925), Vol 2, p 622

27. Eliaser, M., and Kondo, B. O.: Electrocardiographic changes associated with acute porphyria, Am. Heart J. 24:696, 1942
28. Eriksen, L.: Acute porphyria: I. Some observations on the solubility, precipitation, fluorescence and absorption spectra of calcium phosphate-adsorbed porphyrin pigments, Scandinav. J. Clin & Lab Invest 3 121, 1951
29. Falk, J. E., and Benson, A.: Separation of uroporphyrin esters I and III by paper chromatography, Biochem J. 55 101, 1953.
29a. Falk, J. E; Dresel, E. I B, and Rimington, C.: Porphobilinogen as a porphyrin precursor, and interconversion of porphyrins, in a tissue system, Nature, London 172 292, 1953
30. Fink, H, and Hoerburger, W.: Über die Ochronose der Schlachttiere II. Isolierung von krystallisiertem Uroporphyrin aus den Knochen· Identitätsbeweis mit Hilfe der ph-Fluorescenzkurven und durch Analyse, Ztschr. physiol. Chem. 202.8, 1931
31. Fischer, H: Über das Urinporphyrin, Ztschr. physiol. Chem 95.34, 1915
32. Fischer, H.: Über das Kotporphyrin, Ztschr. physiol. Chem 96.148, 1915
33. Fischer, H· Beobachtungen am frischen Harn und Kot von Porphyrinpatienten, Ztschr. physiol. Chem 97:148, 1916
34. Fischer, H. Über die Giftigkeit, die sensibilisierende Wirkung und das spekt. Verhalten der naturlichen Porphyrine, Ztschr physiol Chem. 97:109, 1916
35. Fischer, H.· Farbstoffe mit Pyrrolkernen, in Oppenheimer, C. (ed.): *Handbuch der Biochemie des Menschen und der Tiere* (2d ed, Jena, Gustav Fischer, 1924), Vol 1, p 351
36. Fischer, H, and Hilger, J Zur Kenntnis der naturlichen Porphyrine. VIII Über das Vorkommen von Uroporphyrin (als Kupfersalz Turacin) in den Turakusvogeln und den Nachweis von Koproporphyrin in der Hefe, Ztschr. physiol Chem 138 49, 1924
37. Fischer, H, *et al* Zur Kenntnis der naturlichen Porphyrine XVIII Chemische Befunde bei einem Fall von Porphyrinurie (Petry), Ztschr physiol Chem. 150 44, 1925
38. Fischer, H, and Hilmer, H Über Koproporphyrin-Synthese durch Hefe und ihre Beeinflussung, Ztschr. physiol Chem 153 167, 1926
39 Fischer, H: Über Porphyrine und ihre Synthesen, Ber deutsch chem. Gesellsch 60 2611, 1927
40 Fischer, H, Platz, K, and Morgenroth, K Synthese von Koproporphyrin III und IV, ein Beitrag zur Kenntnis der Porphyrie, Ztschr physiol Chem, 182 265, 1929
41. Fischer, H Über Blut-, Blatt-, und Gallenfarbstoffe, in Oppenheimer, C (ed). *Handbuch der Biochemie des Menschen und der Tiere*, Erganzungsband, p 72 (2d ed, Jena Gustav Fischer, 1930)
42. Fischer, H, and Duesberg, R Über Porphyrine bei klinischer und experimenteller Porphyrie, Arch exper Path u Pharmakol 166 95, 1932
43 Fischer, H· Über Hamin und Porphyrine, Verhandl deutsch. Gesellsch. inn Med 45 7, 1933
44 Fischer, H, and Libowitzky, K Auftreten von Uro-bzw. Koproporphyrin I bei akuter Porphyrie, Ztschr physiol Chem 241.220, 1936.

81. Lyons, H. A . Acute porphyria with spinal fluid changes, Ann Int. Med. 33 711, 1950
82 Macgregor, A G ; Nicholas, R E H , and Rimington, C Porphyria cutanea tarda, Arch Int. Med 90 505, 1952
83 Mackey, L , and Garrod, A E : A further contribution to the study of congenital porphyrinuria (haematoporphyria congenita), Quart J Med 19 357, 1926
84. McSwiney, R R ; Nicholas, R. E H , and Prunty, F. T G Further studies on the porphyrins of acute porphyria, Biochem J , 44 xx, 1949.
84a de Marval, L , and Pons, R Concomitancia entre una porfirinuria congénita e ictericia hemolítica Esplenectomía, Arch argent pediat 5 220, 1934
85 Mason, V R , Courville, C , and Ziskind, E · The porphyrins in human disease, Medicine 12 355, 1933
86 Mertens, E · Über die bei akuter Porphyrie auftretenden Porphyrine, Ztschr. physiol Chemie 250 57, 1937
87. Micheli, F , and Dominici, G Über zwei Falle von familiarer Porphyrie mit letalem Ausgang, Deutsches Arch klin Med 171 154, 1931.
88 Muller, A H Beitrag zur Kenntnis der Porphyria congenita Gunther, Ztschr klin Med 127 460, 1934
89 Nencki, M , and Sieber, N . Über das Hamatoporphyrin, Arch exper Path u Pharmakol 24 430, 1888
90 Nesbitt, S , and Watkins, C H . Acute porphyria, Am J M Sc 203 74, 1942
91 Nesbitt, S Acute porphyria, J A M A 124·286, 1944
91a. Neuberger, A , and Scott, J J Aminolaevulinic acid and porphyrin biosynthesis, Nature, London 172 1093, 1953
92 Nicholas, R E H , and Rimington, C Isolation of unequivocal uroporphyrin III A further study of turacin, Biochem J 49 xxxiii, 1951
93 Nicholas, R E H Chromatographic methods for the separation and identification of porphyrins, Biochem J 48 309, 1951
94 Nicholas, R E H , and Rimington, C Paper chromatography of porphyrins Some hitherto unrecognized porphyrins and further notes on the method, Biochem J 48 306, 1951
95 Nicholas, R E H , and Rimington, C Studies on the Waldenstrom porphyrin of acute porphyria urines Biochem J 55 109, 1953
95a Olson, O C , and Stiles, M H Personal communication
96 Oltman, J E , and Friedman, S Acute porphyria Report of a case showing ineffectiveness of ACTH, New England J Med 244 173, 1951.
96a Paul, K G , Engstrom, A , and Engfeldt, B Localization of porphyrins in bone tissue in a case of porphyria cutanea tarda, J Clin Path 6 135, 1953
97. Pimenta de Mello, R Effect of rose bengal and ultra violet light on porphyrin excretion in rabbits Proc Soc Exper Biol & Med 72 292, 1949
98 Pimenta de Mello, R Effect of light on urinary coproporphyrin excretion in lead-poisoned rabbits, Proc Soc Exper Biol & Med 76 823, 1951
99 Pimenta de Mello, R Effect of Vitamin B_{12}, folic acid, and nicotinamide on urinary coproporphyrin excretion in photosensitized rabbits, Proc Soc Exper. Biol & Med 77 744, 1951

63. Hammond, R. L., and Welcker, M. L.: Porphobilinogen tests on a thousand miscellaneous patients in a search for false positive reactions, J. Lab. & Clin. Med. 33 1254, 1948.
64. Hawkinson, V. E., and Watson, C. J: The separation of porphobilinogen and an Ehrlich negative precursor of uroporphyrin, Science 115 2992, 1952
65 Herbert, F K.: Precursors of porphyrin in the urine in idiopathic porphyria, Biochem J. 52 xii, 1952.
66 Hoppe-Seyler, F.. Cited by Gunther (60).
66a Jope, E M, and O'Brien, J. R P.: Spectral absorption and fluorescence of coproporphyrin isomers I and III, Biochem. J. 39.239, 1945.
67. Jørgensen, J, and With, T
nyere Synspunkter samt et
melse og Provokationsfor
diseases. Review of recent
tive porphobilinogen determination and provocative tests with barbiturates), Nord med. 27 1341, 1945.
68 Kammerer, H, and Meyer, W. K. Über abdominale idiopathische Porphyrie, Deutsches Arch klin Med 179.392, 1936.
69. Kench, J E, and Wilkinson, J F. Porphyrin formation by yeast, Nature, London 155 579, 1945
70 Kenr
ular
71 Kenr
porphyrins on the basis of their x-ray diffraction patterns, Biochem. J in press.
72 Kluver, H. Functional Differences between the Occipital and Temporal Lobes, in Jeffress, L A. (ed) Cerebral Mechanism in Behavior The Hixon Symposium (New York John Wiley & Sons, Inc., 1951).
73. Kvasnickova, V. Influence of folic acid on porphyria, Časop. lék. česk, 87.633, 1947
74. Larjanko, J. Klinisch-pathologische Untersuchungen uber die Porphyria idiopathica abdominalis, Acta Soc med fenn "Duodecim" (Ser B), Vol. 21, fasc 2, 1935
75 Larson, E A, and Watson, C J Studies of coproporphyrin II An investigation of the contribution to the urinary coproporphyrin of hemoglobin and of bacterial metabolism in the intestinal tract, J. Clin Invest 28 452, 1949
76 Linder, A C Salt metabolism in acute porphyria: Report of 2 cases, J A M A. 136 427, 1948
77 London, I M, et al Porphyrin formation and hemoglobin metabolism in congenital porphyria, J Biol Chem 184 365, 1950
78. Lopez Garcia, A. Personal communication
79 Lowry, P. T, Hawkinson, V, and Watson, C J An isotopic study of type III porphyrins and hemoglobin metabolism in an unusual case of "mixed" porphyria, Metabolism 1 149, 1952
80. Lups, S, and van Dijk, C P Riboflavinetoediening bij porphyrie met gunstige uitkomsten [Favorable effects of riboflavin administration in porphyria] Nederl tijdschr geneesk 94 1720, 1950

81 Lyons, H A. Acute porphyria with spinal fluid changes, Ann Int Med. 33 711, 1950
82 Macgregor, A. G, Nicholas, R. E. H, and Rimington, C. Porphyria cutanea tarda, Arch Int Med 90 505, 1952
83 Mackey, L, and Garrod, A E.. A further contribution to the study of congenital porphyrinuria (haematoporphyria congenita), Quart J Med 19 357, 1926
84 McSwiney, R R, Nicholas, R E. H, and Prunty, F T G Further studies on the porphyrins of acute porphyria, Biochem J, 44 xx, 1949
84a de Marval, L, and Pons, R Concomitancia entre una porfirinuria congénita e ictericia hemolitica Esplenectomía, Arch argent pediat 5 220, 1934
85 Mason, V. R, Courville, C, and Ziskind, E. The porphyrins in human disease, Medicine 12 355, 1933
86 Mertens, E Über die bei akuter Porphyrie auftretenden Porphyrine, Ztschr. physiol Chemie 250 57, 1937
87. Micheli, F, and Dominici, G Über zwei Falle von familiarer Porphyrie mit letalem Ausgang, Deutsches Arch klin Med 171 154, 1931
88. Muller, A H Beitrag zur Kenntnis der Porphyria congenita Gunther, Ztschr klin. Med 127 460, 1934
89 Nencki, M, and Sieber, N Über das Hamatoporphyrin, Arch exper Path. u Pharmakol 24 430, 1888
90 Nesbitt, S, and Watkins, C H Acute porphyria, Am J M. Sc 203 74, 1942
91. Nesbitt, S Acute porphyria, J A M A 124 286, 1944
91a Neuberger, A, and Scott, J J · Aminolaevulinic acid and porphyrin biosynthesis, Nature, London 172 1093, 1953
92 Nicholas, R E H, and Rimington, C Isolation of unequivocal uroporphyrin III A further study of turacin, Biochem J 49 xxxiii, 1951
93 Nicholas, R E. H Chromatographic methods for the separation and identification of porphyrins Biochem J 48 309, 1951
94 Nicholas, R E H, and Rimington, C Paper chromatography of porphyrins Some hitherto unrecognized porphyrins and further notes on the method, Biochem J 48 306, 1951
95 Nicholas, R E H, and Rimington, C Studies on the Waldenstrom porphyrin of acute porphyria urines Biochem J, 55 109, 1953
95a Olson, O C, and Stiles, M H Personal communication
96 Oltman, J E, and Friedman, S Acute porphyria Report of a case showing ineffectiveness of ACTH, New England J Med 244 173, 1951
96a Paul, K G, Engstrom, A, and Engfeldt, B Localization of porphyrins in bone tissue in a case of porphyria cutanea tarda, J Clin Path 6 135, 1953
97. Pimenta de Mello, R Effect of rose bengal and ultra violet light on porphyrin excretion in rabbits Proc Soc Exper Biol & Med 72 292, 1949
98 Pimenta de Mello, R Effect of light on urinary coproporphyrin excretion in lead-poisoned rabbits, Proc Soc Exper Biol & Med 76 823, 1951
99 Pimenta de Mello, R Effect of Vitamin B_{12}, folic acid, and nicotinamide on urinary coproporphyrin excretion in photosensitized rabbits, Proc Soc Exper. Biol & Med 77.744, 1951.

100. Prunty, F. T. G. Acute porphyria: Some properties of porphobilinogen, Biochem J. 39 416, 1945
101. Prunty, F. T. G.. Acute porphyria: I. Investigations on pathology of porphyrins and identification of excretion of uroporphyrin I, Arch Int. Med 77:623, 1946.
102. Prunty, F. T. G.: Sodium and chloride depletion in acute porphyria with reference to the status of adrenal cortical function, J. Clin. Invest. 28.690, 1949.
103. Rentschler, E. H.: Porphyria and its simulation of surgical conditions, Am. Surgeon 18.758, 1952.
104 Reithinger, K, and Klee, P.· Zur biologischen Wirkung der Porphyrine, Arch exper Path. u Pharmakol. 127:277, 1928.
105. Rimington, C. Some cases of congenital porphyrinuria in cattle. Chemical studies upon the living animals and post-mortem material, Onderstepoort J Vet. Sc. 7.567, 1936.
105a. Rimington, C: An enzymic theory of haemopoiesis, Compt rend Trav lab Carlsberg, Ser chim 22.454, 1938.
106. Rimington, C. A reinvestigation of turacin, the copper porphyrin pigment of certain birds belonging to the Musophagidae, Proc. Roy. Soc. London 127:106, 1939.
107 Rimington, C., and Miles, P. A. A study of the porphyrins excreted in the urine by a case of congenital porphyria, Biochem. J 50 202, 1951
108 Rimington, C Haems and porphyrins in health and disease, Acta med scandinav. 143 161 and 177, 1952.
108a Rimington, C. Personal communication.
109 Sachs, P Ein Fall von akuter Porphyrie mit hochgradiger Muskelatrophie, Klin. Wchnschr. 13·1123, 1931.
110. Salzburg, P., and Watson, C J · A study of the supposed conversion of protoporphyrin to coproporphyrin by the liver II The porphyrin metabolism of rabbit liver, J Biol Chem 139 593, 1941
110a Salomon, K, Richmond, J E., and Altman, K I Tetrapyrrole precursors of protoporphyrin IX I Uroporphyrin III, J Biol Chem 196.463, 1952.
111 Schmid, R, Schwartz, S, and Watson, C J Porphyrins in the bone marrow and circulating erythrocytes in experimental anemias, Proc Soc. Exper Biol & Med 75 705, 1950
112. Schmid, R, Hanson, B, and Schwartz, S. Experimental porphyria I Isolation of uroporphyrin I from bone marrow of lead poisoned rabbits, Proc Soc Exper Biol & Med 79 459, 1952
113 Schmid, R, and Schwartz, S Experimental porphyria III Hepatic type produced by sedormid, Proc Soc Exper Biol & Med 81 685, 1952
114. Schmid, R, and Schwartz, S Disturbance of catalase metabolism in experimental porphyria. J Lab & Clin Med 40 939, 1952.
115 Schmid, R, Schwartz, S, and Watson, C J The porphyrin content of bone marrow and liver in the various forms of porphyria, A M A Arch Int Med 93:167, 1954
116. Schmid, R; Schwartz, S, and Watson, C J Neuere Ergebnisse auf dem Gebiete der Porphyrine, Acta haematol 10 150, 1953
117. Schmid, R, and Schwartz, S To be published

118 Schmid, R : To be published
119 Schmidt, P. R.. Neurologische und psychische Storungen bei Porphyrinkrankheiten, Fortsch Neurol u Psychiat. 20 422, 1952
120. Schwartz, S , and Watson, C. J : Isolation of uroporphyrin from the feces in idiopathic porphyria, Proc. Soc. Exper Biol. & Med 47·390, 1941.
120a Schwartz, S , et al . A micromethod for the quantitative determination of the urinary coproporphyrin isomers (I and III), J. Biol. Chem. 168 133, 1947.
121. Schwartz, S ; Keprios, M., and Schmid, R Experimental porphyria: II Type produced by lead, phenylhydrazine and light, Proc. Soc. Exper Biol & Med 79 463, 1952
122 Shemin, D , and Rittenberg, D The utilization of glycine for the synthesis of a porphyrin, J. Biol. Chem 159.567, 1945.
123 Shemin, D , and Wittenberg, J The mechanism of porphyrin formation The role of the tricarboxylic acid cycle, J Biol Chem 192.315, 1951
124 Shemin, D , and Kumin, S · The mechanism of porphyrin formation The formation of a succinyl intermediate from succinate, J Biol Chem 198 827, 1952
124a. Shemin, D, and Russell, C S δ-Amino levulinic acid. Its role in the biosynthesis of porphyrins, J Am Chem Soc. 75 4873, 1953
125 Stich, W , Die Bedeutung der B_2 Vitamine fur den Dualismus der Porphyrine und den Aufbau von Haminproteiden. Deutsche med Wchnschr 75 1217, 1950
126 Stich, W , and Eisgruber, H Steuerung der biologischen Porphyrinsynthese und Katalyse der Haminproteidbildung durch Lactoflavin, Klin Wchnschr 28 133, 1950
127. Stokvis, B J Zur Pathogenese der Hamatoporphyrinurn, Ztschr klin Med. 28 1, 1895
128 Sveinsson, S L , Rimington, C , and Barnes, H D Complete porphyrin analysis of pathological urines, Scandinav J Clin & Lab Invest 1 2, 1949
129 Tappeiner, S Die bullose Porphyrindermatose bei Erwachsenen, Wien klin Wchnschr 65(n f 8) 143, 1953
130 Taylor, J. J , et al Chronic porphyria, J A M A 131·26, 1946
131 Turner, W J , and Obermayer, M E Studies on porphyria II A case of porphyria accompanied with epidermolysis bullosa, hypertrichosis and melanosis, Arch Dermat & Syph 37 549, 1938
132 Turner, W J A theory of porphyrinogenesis, J Lab. & Clin Med 26 323, 1940.
133 van Berckel, G J J , Porphyrieen en porphyrinen, Geneesk. bl klin en lab prakt 25 1, 1926
134 van den Bergh, A A H , Regniers, and Muller Ein Fall von kongenitaler Porphyrinurie mit Koproporphyrin in Harn und Stuhl, Arch. Verdauungskr 42 302, 1928
135 van den Bergh, A A H , Muller, P., and Hymans, A : On Porphyrinmodalities, Proc K Akad van wetenschappen te Amsterdam 32 1, 1929
136 van den Bergh, A A H ; Grotepass, W., and Revers, F E Beitrag uber das Porphyrin in Blut und Galle, Klin Wchnschr 37·1534, 1932.

137 van den Bergh, A. A H., und Grotepass, W.: Porphyrinämie ohne Porphyrinurie, Klin Wchnschr. 15:586, 1933.
138 Vannotti, A.. *Porphyrine und Porphyrinkrankheiten* (Berlin Julius Springer, 1937).
139 Verlingstad, H.: Prostigmine in porphyria. Nord. med 42.1432, 1949.
140. Waldenstrom, J: Untersuchungen über Harnfarbstoffe, hauptsachlich Porphyrine, mittels der chromatographischen Analyse, Deutsches Arch Klin Med. 178.38, 1935
141. Waldenstrom, J, Fink, H., and Hoerburger, W.: Über ein neues bei der akuten Porphyrie regelmassig vorkommendes Uroporphyrin, Ztschr physiol Chem 233 1, 1935.
142. Waldenstrom, J. Studien über Porphyrie, Acta med. scandinav, supp 82, 1937.
143. Waldenstrom, J Neurologic symptoms caused by so-called acute porphyria, Acta psychiat et neurol 14 375, 1939.
144 Waldenstrom, J, and Vahlquist, B: Studien uber die Entstehung der roten Harnpigmente (Uroporphyrin und Porphobilin) bei der akuten Porphyrie aus ihrer farblosen Vorstufe (Porphobilinogen), Ztschr. physiol Chem 260 169, 1939.
145. Waldenstrom, J, and Wendt, S Tierexperimentelle Studien uber den Porphyrinstoffwechsel, Ztschr physiol. Chem. 259.157, 1939
146 Waldenstrom, J, and Vahlquist, B Studies on the excretion of porphobilinogen in patients with so-called acute porphyria, Acta med scandinav. 117·1, 1944.
147 Watson, C J, and Schwartz, S. The excretion of zinc uroporphyrin in idiopathic porphyria, J Clin Invest 20 440, 1941
148 Watson, C J, Pass, I J and Schwartz, S. A study of the supposed conversion of protoporphyrin to coproporphyrin by the liver I. The fate of parenterally administered protoporphyrin in dogs with bile renal fistulae, J Biol Chem. 139 583, 1941
149 Watson, C J, and Schwartz, S A simple test for urinary porphobilinogen, Proc. Soc Exper Biol & Med 67 393, 1941
150 Watson, C J, Schwartz, S, and Hawkinson, V Studies of the uroporphyrins. II Further studies of the porphyrins of the urine, feces, bile, and liver in cases of porphyria, with particular reference to a Waldenstrom type porphyrin behaving as an entity on the Tswett column, J Biol Chem 157 345, 1945
151 Watson, C J, and Larson, E A The urinary coproporphyrins in health and disease, Physiol Rev 27 478, 1947
152. Watson, C J, *et al* Studies of coproporphyrin III. Idiopathic coproporphyrinuria, a hitherto unrecognized form characterized by lack of symptoms in spite of the excretion of large amounts of coproporphyrin, J Clin. Invest 28.465, 1949
153 Watson, C. J The erythrocyte coproporphyrin, Arch Int Med. 86 797, 1950
154. Watson, C J Some recent studies of porphyrin metabolism and porphyria, Lancet 1.539, 644, 1951

155 Watson, C J, *et al.* Porphyrin chromogens or precursors in urine, blood, bile, and feces, J Lab & Clin Med. 37 831, 1951
156 Watson, C. J, and Larson, E. A The Porphyrins and Their Relation to Diseases. Porphyria, in *Oxford Medicine* (New York. Oxford University Press, 1951), Vol. IV, Pt II, p 228
157 Watson, C J, *et al* · The manifestations of the different forms of porphyria in relation to chemical findings, Tr. A. Am Physicians 64 345, 1951.
158 Watson, C J. Porphyrin Metabolism, in Duncan, G. G, (ed). *Diseases of Metabolism* (3d ed, Philadelphia· W. B Saunders Company, 1952).
159 Watson, C. J; Schwartz, S, and Schmid, R · Experimental porphyria with special reference to its implications for the human disease, Tr. Am Clin & Climatol. A. 64 105, 1952
160 Watson, C. J, Hawkinson, V, and Bossenmaier, I: Some studies of the nature and clinical significance of porphobilinogen, Tr. A. Am. Physicians 66 144, 1953.
161. Watson, C. J; Hawkinson, V, and Bossenmaier, I. Studies of porphobilinogen, Arch Int Med. In press
162 Wehrmacher, W. H. New symptomatic treatment for acute intermittent porphyria, Arch Int Med 89 111, 1952
163 Westall, R C Isolation of porphobilinogen from the urine of a patient with acute porphyria, Nature 170 614, 1952
164 Zuelzer, W W., and Kaplan, E: Personal communication.

137. van den Bergh, A A H, und Grotepass, W.: Porphyrinamie ohne Porphyrinurie, Klin. Wchnschr. 15.586, 1933.
138 Vannotti, A.: *Porphyrine und Porphyrinkrankheiten* (Berlin Julius Springer, 1937).
139 Verlingstad, H.: Prostigmine in porphyria Nord. med 42,1432, 1949.
140 Waldenstrom, J Untersuchungen uber Harnfarbstoffe, hauptsachlich Porphyrine, mittels der chromatographischen Analyse, Deutsches Arch. klin Med. 178 38, 1935.
141. Waldenstrom, J; Fink, H., and Hoerburger, W.: Über ein neues bei der akuten Porphyrie regelmassig vorkommendes Uroporphyrin, Ztschr. physiol Chem. 233 1, 1935
142. Waldenstrom, J Studien uber Porphyrie, Acta med scandinav, supp 82, 1937.
143. Waldenstrom, J., Neurologic symptoms caused by so-called acute porphyria, Acta psychiat. et neurol 14 375, 1939.
144. Waldenstrom, J., and Vahlquist, B. Studien uber die Entstehung der roten Harnpigmente (Uroporphyrin und Porphobilin) bei der akuten Porphyrie aus ihrer farblosen Vorstufe (Porphobilinogen), Ztschr physiol Chem 260 189, 1939
145. Waldenstrom, J, and Wendt, S. Tierexperimentelle Studien über den Porphyrinstoffwechsel, Ztschr physiol Chem 259.157, 1939
146 Waldenstrom, J., and Vahlquist, B., Studies on the excretion of porphobilinogen in patients with so-called acute porphyria, Acta med. scandinav 117 1, 1944
147 Watson, C J, and Schwartz, S The excretion of zinc uroporphyrin in idiopathic porphyria, J Clin Invest 20 440, 1941.
148 Watson, C J, Pass, I J, and Schwartz, S A study of the supposed conversion of protoporphyrin to coproporphyrin by the liver I The fate of parenterally administered protoporphyrin in dogs with bile renal fistulae, J. Biol. Chem 139 583, 1941
149 Watson, C J, and Schwartz, S A simple test for urinary porphobilinogen, Proc. Soc Exper Biol & Med 67 393, 1941
150 Watson, C J, Schwartz, S, and Hawkinson, V Studies of the uroporphyrins II Further studies of the porphyrins of the urine, feces, bile, and liver in cases of porphyria, with particular reference to a Waldenstrom type porphyrin behaving as an entity on the Tswett column, J Biol Chem 157 345, 1945
151 Watson, C J, and Larson, E. A The urinary coproporphyrins in health and disease, Physiol Rev 27 478, 1947
152 Watson, C J, *et al* Studies of coproporphyrin· III Idiopathic coproporphyrinuria, a hitherto unrecognized form characterized by lack of symptoms in spite of the excretion of large amounts of coproporphyrin, J Clin Invest 28 465, 1949
153. Watson, C J The erythrocyte coproporphyrin, Arch Int Med 86 797, 1950
154 Watson, C J Some recent studies of porphyrin metabolism and porphyria, Lancet 1:539, 644, 1951

Diaphragmatic Hernia

SYDNEY WEINTRAUB

New York Hospital-Cornell University Medical Center, New York

THE CLINICAL significance of diaphragmatic hernia, particularly the hiatal variety, has come to the fore in recent years Daily, patients are referred to the Department of Radiology at the New York Hospital for examination for this condition, and not infrequently the clinician specifies that the examiner place the patient in the Trendelenburg position. The internist, the gastroenterologist, and the cardiologist have become aware that the symptoms produced by hiatus hernia may simulate many of the diseases found in the upper abdomen and chest. They therefore look for hiatus hernia when the patient has an unexplained microcytic hypochromic anemia, when anginal pain is present without any objective evidence of coronary artery disease, or when the recumbent position aggravates the patient's digestive disturbances. Furthermore, it is now well understood that the complications of this condition may be extremely serious and that early diagnosis may be lifesaving

HISTORY—Ambrose Paré (38) was apparently the first to describe diaphragmatic hernia, when in 1575 he reported 2 cases of traumatic origin, one involved the stomach and the other the colon The diagnoses were made at necropsy Hildanus (24), in 1608, reported a case of diaphragmatic hernia discovered at postmortem examination The man had received a sword wound in the chest, had apparently recovered, but 3 months later died of an acute attack of chills, fever, and "black" vomiting The entire stomach had herniated into the chest In 1656, Riverius (43) documented the first case of congenital hernia which he discovered at autopsy in a 24 year old man. Morgagni (33), in 1769, described a traumatic diaphragmatic hernia in a man who

The incidence of diaphragmatic hernia varies, depending on the report, from 1 to 12 per cent. Schatzki (47) reported an incidence of 3 5 per cent in 1,500 roentgenographic examinations of the gastrointestinal tract. Nuzum (34) found 12 27 per cent in 957 patients. Rigler and Emboe (41) concluded that 18 per cent of women in the last stages of pregnancy have hiatus hernias. Johnstone (25) and Pickard (39a) *have reported an incidence of* 11 *per cent and it is their* belief that the use of the Trendelenburg position is responsible for *this high incidence.*

On the other hand, Kirklin and Hodgson found that the occurrence of diaphragmatic hernia was a little over 1 per cent. Evans (17a) [illegible] 6,124

New

The following factors may perhaps explain why there are differences in the reported incidence of diaphragmatic hernia (*1*) In most large hospitals, where the volume of work is large, the radiologist does not routinely examine each patient for the presence of a hiatus hernia, since a special, time-consuming technic is required. It is only employed when a hernia is suspected by the clinician or when the behavior of the cardia deviates from the normal during the fluoroscopic examination (2) As emphasized by Johnstone (25) and Jones (27), a hiatus hernia may be demonstrated at one examination and not visualized at a second, so that two or three examinations may be necessary to establish the presence of the hernia. (3) Although the majority of hiatal hernias are easily diagnosed, in an appreciable group it is difficult to say whether the collection of barium above the esophageal hiatus represents a dilated phrenic ampulla or a sliding hernia There is no doubt that the phrenic ampulla is often incorrectly diagnosed as a hiatus hernia.

EMBRYOLOGY

The embryologic development of the diaphragm is well described by several authors (4, 28, 29) The process is quite complicated and certain phases are still unexplained The completed diaphragm owes its derivation to four sources.

1. The septum transversum (ventral mesentery), which is composed of mesoderm and acts as an early but incomplete diaphragm It develops into the anterior and central portions of the membranous diaphragm, including the segment beneath the heart.

had suffered severe precordial pain. Petit (39), in 1790, reported 2 cases. In 1, there had been complaints of "stomach colic," difficulty in breathing, nausea and vomiting for 40 years; autopsy disclosed a hole in the diaphragm 1 × 2 in. through which the fundus of the stomach, the colon, and the omentum had herniated. A sac was not found, however. The exact point of the rupture is not mentioned. In the second case, the hernia was located on the left side and contained the greater part of the stomach and the entire omentum This patient suffered from attacks of dyspnea and was treated for asthma. Bright (51), in 1836, reported the first case of a congenital short esophageal hernia in a 19 year old girl and called it "misplacement of the stomach."

In our own times, the first comprehensive report and fairly accurate classification of this condition was made by Akerlund (1) in 1926. He found 60 cases in the literature, and added 24 cases of his own. In the same year, Öhnell (34a) reported on 24 collected cases, of which 10 had died of strangulation and 2 of inanition. Thereafter there was no dearth of published material on the subject. But it was not until 1940 that Guthrie and Jones (19) discovered their true clinical significance, they stated that smaller hernias may cause more symptoms than the larger variety.

INCIDENCE

From 1908 through 1925, 30 cases of diaphragmatic hernia were diagnosed clinically at the Mayo Clinic, whereas from 1926 to 1941 this condition was diagnosed in 600 cases, and of these 295 were treated by surgery (21). Although Akerlund had made a classification of hiatus hernia as far back as 1926 and his work was confirmed by Berg (9) and Barsony (8), nevertheless in 1936 Barclay (6), one of the foremost English radiologists, devoted less than a page to the subject, adding the following note of uncertainty. "Some authorities believe that the cardiac portion of the stomach and the esophagus can herniate through the esophageal orifice of the diaphragm when the musculature becomes weakened by old age or some other cause" A few years earlier, Sauerbruch, Chaoul, and Adam (46) had doubted whether the condition of hiatus hernia existed at all and suggested that the diagnosis was the result of a misinterpretation of the roentgenograms which actually showed a dilated phrenic ampulla.

the circumference of the thorax It is re-enforced by two musculotendinous structures, the crura (Fig 1). The right crus arises from the first, second, and third lumbar vertebrae on the right and runs upward to the left so that its left border lies in front of the aorta It then divides and completely encircles the esophagus The left crus is smaller, varies in its attachments, and is of no importance in hiatus hernia Additional anatomic data will be found in the discussion of the causes of the various types of hiatus hernia and their surgical repair.

PHYSIOLOGIC MECHANISM OF THE CARDIA

It has been well established that there is no muscular sphincter at the cardia Lendrum (30) made an anatomic study of 150 cases of cardiospasm and found no evidence of a sphincter. Allison, in explaining the sphincter-like action at the cardia, believes it is dependent on the following factors. (1) the course taken by the lower end of the esophagus, which turns sharply forward and to the left, entering the stomach at an acute angle, (2) the fact that the right crus of the diaphragm acts as a sling and compresses the esophagus at the esophagogastric junction, (3) action by the circular muscle fibers of the esophagus and the oblique fibers of the stomach as an intrinsic mechanism

Barrett (7), on the other hand, believes that although the right crus may have a pinchcock action on the cardia during inspiration, it is not the sole factor which prevents reflux. As proof he cites the fact that paralysis of the diaphragm does not result in regurgitation In his opinion, the acute angle between the esophagus and stomach is maintained by the action of certain muscle bundles in the stomach itself There are longitudinal bands of muscle beneath the gastric mucosa adjacent to the lesser curvature running upward on the posterior wall, encircling the cardia, and then coursing downward on the anterior wall, thus forming a sling around the cardia. As long as the acute angulation between the esophagus and stomach exists, as in paraesophageal hernia, the contraction of these intrinsic muscle fibers will prevent reflux When the acute angulation no longer obtains, as in the sliding hernia, these muscles are powerless to prevent reflux.

CLASSIFICATION

Kirklin and Hodgson (29) state that there are almost as many classifications as there are writers on the subject. They use Harring-

2. The median dorsal part of the membranous diaphragm, which is derived from the dorsal mesentery.

3. The lateral portions posteriorly, which are derivatives of the pleuroperitoneal membranes.

4. A small portion of the membranous diaphragm in the costal regions, bilaterally, which originates from the thoracic wall.

The striated muscle of the [illegible] caudally with the septum transversum to form the dorsal muscular elements of the diaphragm The lateral body walls also furnish derivatives to form muscular components of the diaphragm. Arey believes that the central tendinous portion is a result of muscle degeneration

In the young embryo the stomach begins to develop in the thorax, and as the diaphragm descends it carries the esophagus and stomach with it. The diaphragm finally fuses around the cardiac end of the esophagus. Failure of the esophagus to elongate or arrest in the descent of the stomach produces the congenital anomaly known as congenital short esophageal hernia or thoracic stomach.

The complicated embryologic development of the diaphragm explains the large variety of congenital defects or weak points. The most important of these are:

1. *Sinus of Morgagni (space of Larrey, anterior substernal space, parasternal space)* —This is located along the inner surface of the chondrocostal margin near the sternum, between the costal and chondral insertions of the diaphragm (Fig. 1).

2 *Foramen of Bochdalek (pleuroperitoneal sinus)* —This defect occurs laterally and posteriorly in the muscular part of the diaphragm, near its costal insertion (Fig. 1).

3. *Esophageal hiatus* —Located to the left of the midline posteriorly, it is the most frequent site at which hernias develop as a result of an actual defect or of progressive weakening of the muscle fibers of the right crus

4. *Absence of hemidiaphragm (Fig 8), or other defect* —Arrested development of any segment of the diaphragm may result in a variety of defects

ANATOMY

The diaphragm is a musculofibrous structure composed of a large central tendon to which are attached the muscle fibers arising from

childhood and have been almost completely forgotten by the patient. The commonest sites for this type of traumatic hernia are the posterior portion of the left leaf of the diaphragm and the dome It may also occur through the esophageal hiatus, through the areas of embryologic fusion, and, rarely, through the right half of the diaphragm. The traumatic hernias through the esophageal hiatus usually have a sac, the others do not. Hernias produced by direct injury may occur anywhere in the diaphragm and are the result of stab or gunshot wounds Of the hernias due to inflammatory necrosis subphrenic abscess is the most common cause Rarely, it may be caused by a drainage tube placed in an empyema cavity

CONGENITAL HERNIA

HERNIA OF SINUS OF MORGAGNI.—Normally, there is only a potential opening at this point, but if a congenital weakness is present a hernia may develop in adult life (6, 7, 49). It is more frequent on the right side of the sternum, usually contains the colon and the omentum, and rarely a portion of the stomach Sweet (49) believes that the abdominal approach for its repair is better than the transthoracic approach

FORAMEN OF BOCHDALEK.—Hernias through this sinus are most common on the left side because the dome of the liver protects the right leaf of the diaphragm The defect is located in the muscular portion of the diaphragm, and the herniated viscera are not enclosed in a peritoneal sac This type of hernia is best repaired through the transthoracic approach and does not present serious difficulties

CONGENITALLY SHORT ESOPHAGUS WITH HIATUS HERNIA (THORACIC [illegible]

the third costal cartilage, and most of the stomach, enclosed by a sac, was above the diaphragm He concluded that this anomaly was due to failure of the esophagus to lengthen so that the stomach could not migrate caudad with the diaphragm, and he considered this to be a true hernia

Fineman and Connor (18) reported the roentgenographic aspects of this condition in a case of their own and collected 5 others from the literature Clerf and Manges (16) described the esophagoscopic, radiographic, and clinical findings in 9 cases.

ton's classification (20) with a few modifications of their own. The one offered here is a combination of Harrington's and Allison's (3) classifications.

NONTRAUMATIC	TRAUMATIC
Congenital	Direct injury
	Indirect injury
	Result of inflammatory necrosis

Esophageal hiatus (congenital type)
Paraesophageal
Sliding
Gaps in diaphragm, or absence of hemidiaphragm
Acquired at esophageal hiatus
Paraesophageal
Paraesophageal with slide
Sliding
Sliding with paraesophageal

DISTRIBUTION.—The hernias through the esophageal hiatus are by far the most common. Kirklin and Hodgson reported an incidence of 98 per cent, Evans, of 89 6 per cent. Harrington found, among 430 patients who underwent surgery, 343 (80 per cent) at the esophageal hiatus, including 12 with congenital short esophagus and 17 with a history of trauma, 9 at the foramen of Bochdalek, and 8 at the foramen of Morgagni. The herniations of the remaining 70 cases were due to congenital defects of the diaphragm, direct and indirect injuries, or necrosis resulting from infection.

TRAUMATIC HERNIA

Harrington (23), who has had the most experience with traumatic hernia, gives its causes as: (1) direct injury, (2) indirect injury, and (3) inflammatory necrosis. Of 67 hernias, he found 54 to be due to indirect injuries, 6 to direct injuries, and 7 to inflammatory necrosis; 65 were through the left side of the diaphragm, 2 through the right The stomach was involved in 66 of these cases, the colon in 59, the small intestine in 38, the spleen in 30, the liver in 20, and the kidney in 2 cases.

With the increase of automobile accidents, the crushing or indirect type of injury has become most frequent. The hernia may develop immediately after the injury or it may not occur until months or years later (15). A history of injury is important. It may have occurred in

lieves that this is a result of the "physiologic changes of age" and is a normal finding in the elderly.

Etiology

Direct cause.—An increase in intra-abdominal pressure, due to obesity, pregnancy, abdominal tumors, coughing, vomiting, straining during defecation, tight corsets, or unaccustomed physical exertion, is the direct cause of esophageal hiatus hernia.

Indirect cause.—Some kind of anatomic or physiologic deficiency at the esophageal hiatus is assumed to be the indirect cause. The phrenoesophageal ligaments, membranous structures attached to the under-surface of the diaphragms and to the lower end of the esophagus (Fig. 4), are presumed to play an important role. Harrington believes that the atrophy of these ligaments, the loose attachment of the peritoneum at the cardia, and an abnormally large hiatus are the principle causes for hiatus hernia. Schatzki (48) thinks that diminished elasticity of the diaphragmatic dome and loss of fat tissue around the hiatus cause relaxation and separation of the muscular elements of the hiatus. He, too, maintains that small hernias are a physiologic finding in the older age groups.

Allison (3), who has had many opportunities to examine the esophageal hiatus at operation, claims that the action of the right crus of the diaphragm is the most important factor in maintaining the normal anatomic relationship of the cardia. He compares the esophagogastric junction to the anorectal junction (Figs. 2, 3). In the latter, continence not only depends on the anal sphincter but also on the action of the puborectalis muscle which forms a sling around the rectum. In the same manner, the fibers of the right crus part around the esophagus to form a sling as it takes a bend forward and to the left. In front of the esophagus these muscle fibers are reinforced by other elements of the diaphragm, thus forming a firm ridge, but posteriorly, where the fibers have parted, a weak spot is present. An increase in intra-abdominal pressure will therefore split the fibers even more and increase the size of the opening.

The contraction of the right crus compresses the walls of the esophagus and also pulls it down, so that an acute angle is formed between the esophagus and the fundus of the stomach. Allison confirmed this at operation by putting a finger into the esophagus through

Kirklin and Hodgson rightly contend that the term "thoracic stomach" should be employed only in reference to this type of congenital hernia. In the literature there are many descriptions of other types of hernia which contain the entire stomach and which are erroneously called "thoracic stomach." The symptoms, which may start at birth, resemble those of sliding hernia.

CONGENITAL GAPS IN DIAPHRAGM AND ABSENCE OF HEMIDIAPHRAGM.—Small hernias of this variety may go undetected. The large ones produce serious symptoms in the infant, such as cyanosis, asphyxia, and evidence of strangulation. They usually occur on the left side and may contain the stomach, small and large intestines, spleen, liver, kidney, and omentum. Diagnosis is made by physical examination and roentgenographically by the presence of air pockets in the chest. Caffey (14) urges immediate surgical treatment because sudden death may occur and because delay will tend to increase the difficulty in restoring the herniated organs into the underdeveloped abdominal cavity. A few of these patients may reach adult life without any symptoms. One such case was recently studied at the New York Hospital. The patient appeared to be in perfect health and has never had any symptoms referable to the cardiorespiratory system or to the gastrointestinal tract.

The patient, a 30 year old woman, was admitted for the purpose of having a small adenoma of the thyroid excised. A preoperative chest film revealed a diffuse density in the lower third of the left lung field and a diagnosis of malignant tumor of the lung was made. Subsequent x-ray examinations, which included a gastrointestinal series and barium enema, showed the colon and small bowel to be in the left thorax up to the level of the clavicle. The stomach was extremely low and reversed in position, with the greater curvature on the right and the duodenum on the left. The gallbladder and liver were in their normal position (Fig. 8).

ACQUIRED ESOPHAGEAL HIATUS HERNIA

This form of hernia is divided into two main groups: the paraesophageal and the sliding type. Akerlund (2) uses the term "acquired" in a different sense. To him it means small sacless protrusions through the esophageal hiatus occurring frequently in elderly people and due to the relaxation of the esophagophrenic ligaments. He be-

reflux of gastric contents does not occur because the esophagus maintains its acute angulation in relation to the stomach. In addition, the filled stomach produces pressure on the adjacent esophagus and thus helps to prevent regurgitation.

Allison believes that in some instances of paraesophageal hernia a "preformed" sac may have remained in the thorax because of a fault in embryologic development and as a result a hernia could develop at any period of life Sweet has found a narrow bundle of muscle fibers separating the hernia from the esophagus, with the esophageal hiatus remaining normal in size. He therefore prefers to call this type of hernia the "parahiatal" type. Although it is generally thought that the sac of the paraesophageal hernia lies anterior to the esophagus, Allison reports a case in which the sac was behind the esophagus, probably formed by the peritoneum of the lesser sac

After a paraesophageal hernia develops, the cardia can slide up into the mediastinum. This is known as paraesophageal hernia with slide. Reflux of stomach contents does not occur in this type because of the acute angulation between the esophagus and the stomach

Sliding Hiatus Hernia (Short Esophagus)

This, by far the most common variety, may produce the most distressing symptoms and complications (Fig. 11). In most large series it comprises about 90 per cent of the cases. As the stomach slides through the hiatus and displaces the esophagus upward into the mediastinum, the esophagus appears to be shortened or may actually become so because of inflammatory changes. Many workers have therefore called it short esophagus hernia. However, it should not be mistaken for the true, congenital short esophageal type.

SYMPTOMATOLOGY

The main complaints are upper abdominal or retrosternal pain, fullness after meals, gaseous distention, vomiting, pressure around the heart, palpitation, tachycardia, dyspnea, and weakness due to anemia. At first, the patient may have a mild sense of epigastric fullness and distress which radiates through to the back; it usually comes on after large meals but may occur immediately after taking a small quantity of food The attacks do not vary much in character but their

the stomach. He observed that the angulation was increased from side to side and from in front backward. He further noted that during [illegible] off the esophagus [illegible] when the suction, [illegible] to do this.

Although the right crus acts as a sling, it does not hug the esophagus tightly nor is it applied at any fixed point or level This allows the intra-abdominal segment of the esophagus to slide up and down. Rios-Solans (42) proved these facts on postmortem examination of 50 adults ranging in age from 31 to 78 years. In 26 per cent, 1 or 2 fingers could be inserted through the esophageal hiatus, and he concluded that a hiatus hernia could easily have developed in these individuals.

Barrett (7) thinks along the same lines. He believes that, potentially, anyone can acquire a hiatus hernia because the hiatus is really a "hole" in the diaphragm. The margins of the hiatus are loosely applied to the esophagus, which has a considerable degree of slide up and down. The phrenoesophageal ligaments, which are tenuous bands of connective tissue, play no part in preventing herniation He offers the novel theory that the left gastric artery plays the important role in preventing herniation of the stomach. The left gastric artery binds the upper portion of the stomach to the posterior wall. This artery has two branches, one supplies the lower esophagus, the other the lesser curvature of the stomach. If the mesentery of the artery is lengthened or if its terminal branches are at some distance from the edge of the lesser curvature, the stomach is loosely attached, with an increase in intra-abdominal pressure, a sliding hernia could then develop.

Paraesophageal (Parahiatal) Hernia

This type of hernia is less common, occurring in 7 to 10 per cent of cases The cardia maintains its normal position, and the esophagus its normal length At first, only the fundus of the stomach is engaged in the hernial sac, but as the hernia grows larger, the entire stomach and omentum is drawn into the sac. The stomach may then rotate on its transverse axis, so that the greater curvature occupies the uppermost position (Figs 9, 10) This is frequently referred to as an upside-down stomach. Although such hernias may become exceedingly large,

Symptom	No of Cases	Symptom	No. of Cases
Pain			
Epigastric.	74	Vomiting or regurgitation	75
Substernal	39	Bleeding	28
Costal margin	32	Dyspnea	21
Back	31	Heartburn	16
Right shoulder	19	Palpitation	10
Left shoulder	14	Dysphagia	4
Arm or hand	12		

Bleeding (occult blood to massive hemorrhage) occurred in 22 of the small hernias and in 6 of the large ones Actually, the patients with the smaller hernias had more symptoms and of a more severe nature than those with the large hernias

COMPLICATIONS

Reflux esophagitis.—This is the most frequent and most serious complication of the sliding hiatus hernia. Olsen and Harrington (35) reported evidence of diffuse ulcerative esophagitis at esophagoscopy in 15 per cent of 149 patients with hiatus hernia. Evans (17a) found an incidence of 1 7 per cent in the sliding type Allison's (3) figures are very high, among 176 patients with sliding hernias, 63 had esophageal ulcers with stenosis and 73 had superficial esophagitis with ulceration, giving an incidence of 77 per cent The reason for such a high incidence may be the fact that all but 19 of his patients had an esophagoscopic examination The superficial inflammatory changes and the superficial ulcerations are not usually demonstrated by roentgenographic examination.

At esophagoscopy, the mucosa appears congested, thickened, and redundant The redundancy due to the shortening of the esophagus may obstruct the instrument slightly At the lower end, the inflammation is more pronounced, the mucosa bleeds easily, and small erosions 2 to 3 mm. in diameter are present. The mucosa between the ulcers is edematous and thickened

Some, for example Johnstone (26), feel that the reflux of acid chyme is not the only cause of esophagitis, for in many persons without hiatus hernia who regurgitate their gastric contents esophagitis does not develop, nor does it develop in the chronic ruminators. Some favor the theory that the presence of islands of gastric mucosa in the esophagus predisposes to peptic ulceration Redish and Kertzner (40) discovered such islands in 6 patients with peptic ulcer of the esopha-

severity depends at times on the amount of stomach that is incarcerated in the hernia and the complications that may have developed.

The pain may become very severe and radiate to the left side of the thorax or to the back between the shoulder blades and the patient may have difficulty in belching and in vomiting. This is due to spasm of the diaphragm and reflex cardiospasm, according to Harrington. The diaphragmatic spasm causes an hourglass contraction of the stomach; as the upper pouch enlarges, it presses on the esophagus, thus interfering with belching and vomiting. Pressure on the heart and mediastinal structures also develops, resulting in tachycardia, palpitation, and dyspnea. Irritation of the diaphragm causes phrenic nerve pain which is referred to the left shoulder.

All of these signs and symptoms are most commonly found in paraesophageal hernia. They can be aggravated by exertion, emotional stress, and the recumbent position. The attacks may last several minutes to several hours. They may recur after each meal or at intervals of weeks or months. It is assumed that between attacks the stomach has descended into the abdominal cavity and the diaphragm is no longer spastic. Increase in the frequency of attacks is probably due to the incarceration of the entire stomach or a part of it

Any of the above-mentioned symptoms may be present with a sliding hernia, in addition, symptoms secondary to inflammatory changes in the esophagus, usually referred to as "reflex-esophagitis," may develop. The patient may have a burning pain of varying intensity behind the lower sternum, radiating upward to the neck and jaw or between the scapulas It may radiate to the precordium, to both shoulders, and down both arms to the hands. There may be pain on swallowing, and a feeling of soreness as the bolus goes down the esophagus, with regurgitation and vomiting of food and acid material As the esophagitis grows more severe, ulceration and stenosis develop and the dysphagia becomes worse (Figs 12 and 13). These symptoms are aggravated or brought on when the patient bends forward or when he assumes the recumbent position All these symptoms may occur in the congenital short esophageal hernia because the same mechanism is at work

Jones's excellent study shows the frequency with which the various symptoms occur Of 128 cases of hiatus hernia with symptoms referable to the hernia, 91 were small (less than 7 cm. in diameter) and most of them were 4 cm or less, 37 were large (over 7 cm). His figures are:

duodenum, which becomes attenuated with marked narrowing of the lumen This may be one of the causes of persistent vomiting. Incarceration may develop rapidly.

In 1 such case recently seen in the New York Hospital, the entire stomach was above the diaphragm and completely rotated on its transverse axis. The patient had persistent vomiting for 2 days prior to hospital admission, but had been well before this incident Roentgenograms taken 1 year previously showed a small paraesophageal hernia 6 cm in diameter

An intermittent or temporary incarceration due to prolonged increase in intra-abdominal pressure may occur in persons without previous symptoms. In 1 patient in whom persistent vomiting developed after a pelvic operation, roentgenographic examination revealed a small sliding hernia. The vomiting ceased as soon as a tight abdominal binder was removed and she was permitted to sit up. In another patient, a severe pain in the retrosternal area suddenly developed on a camping trip Having been assigned the task of washing the dishes, he spent almost an hour, after a full meal, squatting and bending forward beside a brook. When he finally straightened up he experienced a sharp pain which was aggravated by the swallowing of food and hot liquids. The pain subsided gradually in the course of a week A small sliding hernia was found by x-ray examination.

DIFFERENTIAL DIAGNOSIS

ANGINA PECTORIS.—The differentiation between the symptoms of hiatus hernia and the angina of coronary artery disease can be extremely difficult, but it is a problem with which the clinician is frequently confronted. The pain in both conditions may be substernal, and it may radiate to the left shoulder and down the arm into the ring and little fingers. In both conditions the pain can be initiated by exertion, overeating, and emotional stress, and relief may be obtained by belching, vomiting, and the use of nitroglycerine Hernias of large size may compress the heart and mediastinal structures, causing palpitation, precordial distress, dyspnea, and cyanosis, but it is the smaller ones that are more apt to produce symptoms simulating angina pectoris

The distribution of pain produced by hiatus hernia is governed by the visceral afferent pathways arising from the esophagus, stomach, and diaphragm. Jones (27), in 1941, made a thorough study of these

gus associated with hiatus hernia, 4 of whom had duodenal ulcers in addition. Rios-Solans, from his anatomic study of 50 cases, concludes that it is not unusual to find gastric mucosal glands lying beneath the stratified esophageal lining membrane. Palmer (37) has studied the esophagogastric junction in 15 normal persons by esophagoscopy and by placing clips at the junction of gastric and esophageal mucosa. On the basis of serial roentgenograms he concluded that a portion of the normal resting abdominal esophagus is lined with gastric mucosa and should be considered as part of the stomach.

Palmer and other investigators (12, 40) are of the opinion that a peptic ulcer may start at the cardia or lower end of the esophagus before there is any evidence of herniation, but as the esophagus contracts due to spasm of the longitudinal fibers it pulls the stomach upward to form a hernia. But this theory is contradicted by all the cases of hiatus hernia which have been followed for years without evidence of ulceration but in which it finally develops. Furthermore, in the primary ulcers of the lower end of the esophagus studied at the New York Hospital, the esophagus became elongated and dilated because of the reflex cardiospasm.

HEMORRHAGE.—Bleeding is a fairly frequent complication. It may be caused by a frank peptic ulcer in the herniated pouch (Fig 14), or it may be due to superficial erosions secondary to the congestion of the gastric mucosa produced by constriction of the vessels at the hiatus. The bleeding may occur as a slow seepage over a long period of time resulting in a secondary anemia without other symptoms, or the hemorrhage can be severe enough to exsanguinate the patient. Of 221 hiatus hernias reported by Sahler and Hampton (45), 14 5 per cent had positive evidence of bleeding. Jones (27) reports an incidence of 21 9 per cent in 128 cases.

INCARCERATION OF HERNIATED VISCERA.—When a portion or the entire stomach becomes incarcerated, the patient presents signs of acute obstruction: severe pain, persistent vomiting, and occasionally hematemesis When the small or large intestines are involved, the signs and symptoms of acute intestinal obstruction are present Rarely the omentum alone may become caught in the sinus of Morgagni (Fig 7). This will cause acute dilatation of the stomach and paralytic ileus. Incarceration of the stomach is due to fixation of the herniated portion secondary to inflammatory changes. When the major portion of the stomach becomes herniated, it tends to rotate on the transverse as well as the longitudinal axis In addition, there is a pull on the

In view of this distribution of the afferent sensory neurons, distention of the lower esophagus or the herniated stomach, with or without inflammatory changes, will obviously produce pain typical of angina pectoris and will also have the same type of radiation Spasm of the diaphragm and irritation caused by the stretching of the hiatus by the distended hernia produces shoulder-pain Jones believes that the vagus

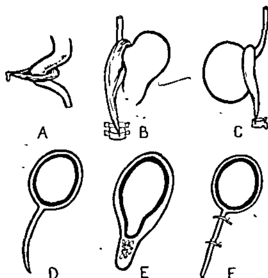


Fig. 2—Schematic drawing of the diaphragm, according to Allison (3) A,
pu
sh
fr
w
fibers behind esophagus

is entirely motor in its function and accounts for an increase in peristalsis and spasm in the esophagus

Master *et al* (32) believe that a hiatus hernia can also produce anginal pain by reflex-vasoconstriction of the coronary arteries, and they quote from the work of Dietrich and Schwiegk (17) who noted a diminution in blood flow through the coronary arteries of dogs when the stomach was distended. They considered this to be due to vagal reflex, since atropinization of vagotomy did not alter the blood flow. Master and Jaffe (31) pointed out that some of the complications of hiatus hernia may cause acute coronary insufficiency. Of these,

pathways, and the following is a summary of the results of his work Pain stimuli originating in the esophagus, stomach, and diaphragm are carried by the visceral afferent neurons and is manifested by viscerocutaneous pain, better known as referred pain. Pain stimuli from the heart travel over the pathways of the first to fifth thoracic segments with a spread to the sixth. Capps (13) found that painful stimuli from the tendinous part of the diaphragm were transmitted by

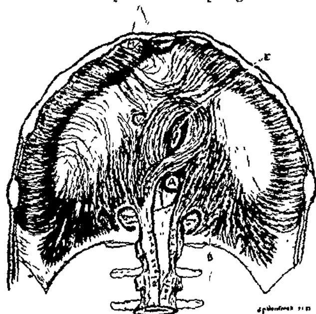


Fig 1 —Schematic drawing of the diaphragm, note that the right crus encircles the esophageal hiatus *A*, aorta, *B*, foramen of Bochdalek, *E*, esophagus, *M*, foramen of Morgagni, *V*, vena cava

the afferent fibers in the phrenic nerve and were referred to the skin areas of the third, fourth, and fifth cervical segments and occasionally to the second cervical segment Therefore, when the tendinous portion of the diaphragm is irritated, pain will be felt in the shoulder and down the arm to the base of the thumb The esophagus is supplied by the fibers coming from the first to sixth thoracic segments and at times by the eighth cervical segment, thus accounting for pain down the forearm and little finger Painful stimuli in the fundus of the stomach are referred anteriorly to the midline in the high epigastric and subxyphoid regions, an area supplied by the seventh, eighth, and ninth thoracic segments.

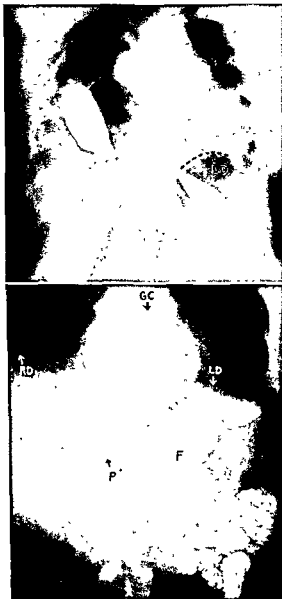


Fig 4 (*above*) —Traumatic hernia due to crushing injury, colon herniated through right side of diaphragm *LD*, normal left side of diaphragm

Fig 5 (*below*) —Herniation of stomach through foramen of Morgagni on the right side, note axial rotation of stomach *F*, fundus, *GC*, greater curvature, *P*, pylorus, *LD*, *RD*, left and right sides of diaphragm.

hemorrhage is the most important, for the drop in hemoglobin is accompanied by anoxemia of the heart muscle. An infarction of the left ventricle may possibly occur. Peripheral vascular shock may develop *after a severe bout of nausea and vomiting, followed by acute* coronary insufficiency.

Bockus (10) also thinks that the vagus nerve plays an important role in
in the
flow, e
periments of Levy, Gilbert, and Russel, who demonstrated that when a patient with coronary disease inhaled a low oxygen mixture anginal

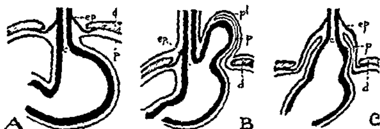


Fig 3.—Diagram toneal reflection at with the sac lying between esophagus phrenoesophageal phrenoesophageal ligaments, *p*, pleura

pain developed and changes appeared in the electrocardiogram. These effects could be brought about more promptly when the stomach was full. The elimination of the vagal influence delayed the appearance of anginal pain

Master in a study of 57 consecutive cases of hiatus hernia, found evidence of organic heart disease in almost two-thirds of the cases Nuzum (34) discovered hiatus hernia in 25 of 100 private patients who suffered with angina pectoris Jones, in his series of 128 cases, had 11 cases of heart disease (8 6 per cent) This difference in incidence may perhaps be explained by the fact that the cardiologist (e g, Master, Nuzum) sees many more patients with coronary artery disease than does the internist, furthermore, these patients have the same habitus and are in the same age group in which hiatus hernia usually occurs

The differential diagnosis between hiatus hernia and coronary

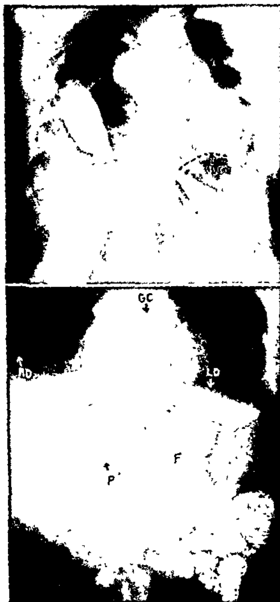


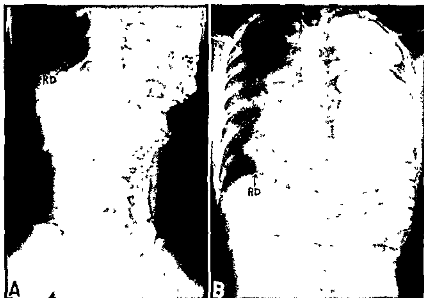
FIG 4 (*above*).—Traumatic hernia due to crushing injury; colon herniated through right side of diaphragm *LD*, normal left side of diaphragm.

FIG 5 (*below*).—Herniation of stomach through foramen of Morgagni on the right side, note axial rotation of stomach *F*, fundus, *GC*, greater curvature, *P*, pylorus, *LD*, *RD*, left and right sides of diaphragm



Fig 6—Omentum incarcerated in a hernia through foramen of Morgagni Roentgen diagnosis was pericardial cyst or lung tumor. *A*, front view *B*, lateral view.

artery disease is based on the following points. (1) Right shoulder pain is much more frequent in hiatus hernia; Jones reported 19 cases of hernia with right shoulder pain and only 1 of coronary disease and hiatus hernia with such pain (2) Fewer patients with hiatus hernia obtain relief from nitroglycerin, nor is its constant use effective in this disease in contrast to its invariable efficacy in angina pectoris (3) Atropine rarely relieves the pain in angina, but frequently does so in



hiatus hernia. (4) Large meals and the recumbent position initiate pain much more frequently in hiatus hernia, whereas exertion and emotional stress consistently produce pain in coronary artery disease Master believes that he can make an accurate differential diagnosis with the 2-step exercise test, some favor the anoxemia test A careful evaluation of the symptoms, and the various tests evaluating the efficiency of the myocardium will usually lead to the correct diagnosis

Gallbladder disease and peptic ulcer —Cholelithiasis and chronic cholecystitis are common in the same age group in which hiatus hernia is found The pain and the digestive disturbances may simulate those

of hiatus hernia and also of coronary artery disease. Here again, a careful evaluation of the history and prolonged observation of the patient usually indicate which one of the pathologic conditions needs the most attention.

Peptic ulcers within the herniated pouch occur, and are probably more frequent than are demonstrated by roentgenographic examinations. They are probably responsible for most of the massive hemor-



Fig. 8 (*left*) —Paraesophageal hernia with slide, note acute angle of esophagus with stomach, cardia has slid up above diaphragm. *C*, cardiac, *H*, herniated pouch, *LD*, left side of diaphragm

Fig 9 (*right*) —Paraesophageal he
volvulus of stomach and deformity of
G, greater curvature, *N*, niche, *LD*,

rhages It is our impression that the incidence of duodenal and pyloric ulcers is higher in patients with hiatus hernia, particularly in paraesophageal variety, than in the rest of the population A disturbed blood supply to the duodenum secondary to the pull exerted upon it by the herniated stomach may be the reason.

The problems in differential diagnosis are well illustrated by the following case.

Mrs. C. R., housewife, age 45, had complained for 15 months of moderately severe, sharp pain high in the epigastrium, left of the midline.

It radiated through to the back and between the scapulas. While she did not think that the pain had any definite relation to meals, small amounts of milk and food relieved the pain for short periods The pain frequently awakened her at night, and vomiting gave her relief. Three months after the onset, her physicians found a normal stomach and duodenum, but the Graham test revealed cholelithiasis. The gallbladder was removed and she was well for 3 months, then the symptoms returned with increasing severity. A second gastrointestinal x-ray series showed a sliding hiatus hernia 5 cm. in diameter.

She was then referred to the New York Hospital for evaluation It was [illegible] e to the hernia and [illegible] scopic examination, [illegible] was applied to the upper abdomen with the hand in an attempt to demonstrate the hernia. This produced considerable pain and the patient volunteered the information *that we had touched the exact spot where she experienced most of the pain.* Further study of this area revealed a niche 1 5 cm. in diameter on the *posterior wall of the upper third of the body of the stomach. The hernia,* which showed clearly on the submitted films, could not be demonstrated at this examination The ulcer has been treated medically with complete relief of symptoms.

Ross and Johnson (44) reported an interesting case which illustrates how sudden and fulminating the symptoms of hiatus hernia may be. Their patient, an obese, 57 year old woman who had never had any symptoms referrable to the cardiorespiratory or gastrointestinal systems, suddenly suffered a severe, sharp pain in the left side of her chest while laughing. This was followed by a 10 minute episode of fainting Incessant vomiting set in, and the patient appeared cyanosed. Roentgenography revealed a paraesophageal hernia containing most of the stomach, with torsion of the antrum. At operation, a sac 8 cm. in diameter was found; it contained the incarcerated stomach and omentum, both of which showed evidence of inflammation and fat necrosis.

Neoplasms and hiatus hernia.—Carcinoma in the herniated portion of the stomach is rare. Among 308 patients with hiatus hernia, Brick (11) found 2 with gastric carcinoma and 1 with esophageal cancer. He concluded that gastric carcinoma is four times as frequent in patients without hiatus hernia as in those with it Pack *et al.* (36) reported 2 cases, they believe that more neoplasms would be found if the diagnoses were more accurate. In our own experience, there has been only 1 case of carcinoma in a herniated stomach.

ROENTGENOGRAPHIC DIAGNOSIS

The roentgenographic demonstration of diaphragmatic hernias is fairly accurate in the majority of cases, but in a small percentage difficulty is encountered. The technics employed are well known. The Trendelenburg position with the Valsalva maneuver has achieved great popularity, but we believe that many more hernias can be demonstrated during the fluoroscopic examination with the patient in the prone and right oblique position Recently it has been recommended that the patient be observed fluoroscopically while bending forward and touching the toes Very few obese patients can accomplish this, and in addition, the visualization is poor.

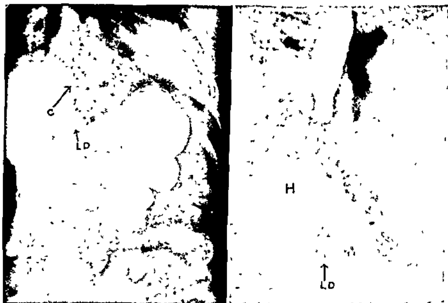


Fig 10 (*left*) —Sliding hernia *C*, cardia, *LD*, left side of diaphragm
Fig 11 (*right*) —Sliding hernia with reflux esophagitis; note stenosis of lower esophagus *H*, herniated pouch, *LD*, left side of diaphragm

A large phrenic ampulla can be mistaken for a hernia. When the ampulla shows a notch on each border it is easily identified (Fig. 14), but this occurs infrequently It is not unusual for several radiologists to disagree whether a hernia is present or not when considering the same set of films. The small protrusions of the stomach through the hiatus in elderly people may cause confusion. Both Akerlund and


N
LD


N


H
LD
N


PH
LD

Schatzki call them "physiologic" in the older age group. Finally, since several examinations may be required to demonstrate a hernia, it is the clinician's duty to request repeated x-ray studies if he strongly suspects the presence of a hernia.

TREATMENT

Medical.—Most patients can be treated medically if the hernia is not too large and if there are no serious complications. The aims of treatment are to prevent overdistention of the herniated portion of the stomach and to keep the acid low. A bland, low roughage diet is therefore recommended, with avoidance of large meals, especially in the evening. Irritants such as alcohol, coffee, and condiments should be avoided. As the majority of these patients tend to be obese, reduction in weight is essential. Strenuous exercise and work which requires much bending or lifting are contraindicated. The patient should learn to sit up straight, particularly after meals and should not lie down after eating. He should sleep on his right side in a semirecumbent position. If there is evidence of hyperchlorhydria it can be controlled by the nonabsorbable alkalis. Atropine and its derivatives will relieve the spasm of the pylorus. It is important that the bowel function be kept normal to prevent abdominal distention and straining. If symptoms of esophageal irritation develop, the patient should be placed on a rigid ulcer regimen with frequent endoscopic examinations to determine the status of the esophageal mucosa. If the inflammatory changes become too severe or if an ulcer has developed, stenosis may occur.

Surgical.—All traumatic hernias and all types of hernias containing the colon or small intestines should be repaired surgically. Harrington believes that if a third or more of the stomach is involved surgery is indicated to prevent possible incarceration. Another indication is the failure of medical treatment

Allison urges surgery in sliding hernia at the first sign of esophagitis because stenosis occurs if this condition is neglected The results of surgical treatment are very poor after stenosis has developed. Allison believes that it is more important to restore the physiologic function of the cardia than to obtain a perfect anatomic result To accomplish this it is necessary to repair the right crus of the diaphragm so that it functions normally (Fig 3)

In recent years, since the thoracic approach has become a much less

formidable procedure, the surgical repair of diaphragmatic hernias has been extremely successful Mortality and morbidity have been low, and recurrences are rare.

Interruption of the left phrenic nerve is a procedure that will sometimes promptly alleviate symptoms. Although it is not a cure, it may be a lifesaving measure, particularly in the elderly, debilitated patient for whom a major surgical procedure is too great a risk

REFERENCES

1 Akerlund, A. Hernia diaphragmatica hiatus esophagei, Acta radiol 6.3, 1926

2 Akerlund, A. Die anatomische Grundlage des Rontgenbildes der sogen "erworbenen Hiatusbruche," Acta radiol 14 523, 1933

3 Allison, P R. Reflux esophagitis, sliding hiatal hernia, and the anatomy of repair, Surg, Gynec & Obst 92 419, 1951.

4 Arey, L. B: *Developmental Anatomy* (Philadelphia W. B Saunders Company, 1942), p 259

5 Bailey, P. A: A case of thoracic stomach, Anat Rec. 17.107, 1919

6 Barclay, A E. *The Digestive Tract* (London Cambridge University Press, 1936), p 219

7 Barrett, N R Discussion on hiatus hernia, Proc Roy Soc Med 45·279, 1952

8 Bársony, T. Ueber die Hiatus-Hernien, Fortsch a d Geb d. Rontgenstrahlen 38 629, 1928

9 Berg, H. H Über das klinische und rontgendiagnostische Bild der Hiatusbruche und der Divertikel des Magendarmkanals, Verhandl Gesellsch Verdauungs- u Stoffwechselkr 10 67, 1930

10 Bockus, H L. Relation of esophageal hiatus to angina pectoris, Gastroenterology 23 325, 1953

11 Brick, I B Hiatus hernia and carcinoma of the stomach and esophagus, Gastroenterology 13 47, 1949

11a Bright, R Account of a remarkable displacement of the stomach, Guy's Hosp Rep 1 598, 1836

12 Buckstein, J *The Digestive Tract in Roentgenology* (Philadelphia J B Lippincott Company, 1948)

13. Capps, J A *An Experimental and Clinical Study of Pain* (New York Macmillan Company, 1932)

14 Caffey, J *Pediatric X ray Diagnosis* (2d ed, Chicago Year Book Publishers, Inc, 1950), p 189

15 Carman, R D, and Fineman, S Roentgenologic diagnosis of diaphragmatic hernia, Radiology 3 26, 1924

16 Clerf, L H, and Manges, W E Congenital anomalies of the esophagus, Ann Otol, Rhin & Laryng 42 1058 1933

17 Dietrich, S, and Schwiegk, K Das Schmerzproblem der Angina pectoris, Klin Wchnschr 12 135, 1933

17a Evans, J A Sliding hernia, Am J Roentgenol 68 754, 1952

Schatzki call them "physiologic" in the older age group. Finally, since several examinations may be required to demonstrate a hernia, it is the clinician's duty to request repeated x-ray studies if he strongly suspects the presence of a hernia.

TREATMENT

MEDICAL.—Most patients can be treated medically if the hernia is not too large and if there are no serious complications. The aims of treatment are to prevent overdistention of the herniated portion of the stomach and to keep the acid low. A bland, low-roughage diet is therefore recommended, with avoidance of large meals, especially in the evening. Irritants such as alcohol, coffee, and condiments should be avoided. As the majority of these patients tend to be obese, reduction in weight is essential. Strenuous exercise and work which requires much bending or lifting are contraindicated. The patient should learn to sit up straight, particularly after meals and should not lie down after eating He should sleep on his right side in a semirecumbent position. If there is evidence of hyperchlorhydria it can be controlled by the nonabsorbable alkalis. Atropine and its derivatives will relieve the spasm of the pylorus. It is important that the bowel function be kept normal to prevent abdominal distention and straining. If symptoms of esophageal irritation develop, the patient should be placed on a rigid ulcer regimen with frequent endoscopic examinations to determine the status of the esophageal mucosa. If the inflammatory changes become too severe or if an ulcer has developed, stenosis may occur.

SURGICAL.—All traumatic hernias and all types of hernias containing the colon or small intestines should be repaired surgically. Harrington believes that if a third or more of the stomach is involved surgery is indicated to prevent possible incarceration. Another indication is the failure of medical treatment

Allison urges surgery in sliding hernia at the first sign of esophagitis because stenosis occurs if this condition is neglected The results of surgical treatment are very poor after stenosis has developed. Allison believes that it is more important to restore the physiologic function of the cardia than to obtain a perfect anatomic result To accomplish this it is necessary to repair the right crus of the diaphragm so that it functions normally (Fig 3)

In recent years, since the thoracic approach has become a much less

41. Rigler, L. G, and Eneboe, J. B. Incidence of hiatus hernia in pregnant women and its significance, J. Thorac. Surg 4 262, 1935
42 Rios-Solans, G.: Sliding hernias through the oesophageal hiatus, Brit M J. 1 1029, 1953.
43 Riverius, L., *Observationes Medicae & Curationes Insignes* (Haage-Comitum A. Vlacq, 1656).
44 Ross, J. R, and Johnson, A. C.· Diaphragmatic hernia: A case report, Gastroenterology 18 448, 1951.
45 Sahler, O D, and Hampton, A. O Bleeding in hiatus hernia, Am J Roentgenol 49.433, 1943.
46 Sauerbruch, F; Chaoul, H., and Adam, A. Anatomisch-klinischer und rontgenologischer Beitrag zur "Hiatushernie," Deutsche med Wchnschr 58 1391, 1932.
47 Schatzki, R · Die Hernien des Hiatus oesophageus, Deutsches Arch f. klin. Med. 173 85, 1932.
48 Schatzki, R. Die Beweglichkeit von Ösophagus und Magen innerhalb des Zwerchschlitzes beim alten Menschen, Fortschr a d. Geb. d Rontgenstrahlen 45 177, 1932.
49 Sweet, R. H: *Thoracic Surgery* (Philadelphia· W. B. Saunders Company, 1951), pp 316–325

18. Fineman, S, and Conner, H. M.: Right diaphragmatic hernia of the short esophagus type, Am J. M. Sc. 167.672, 1924
19. Guthrie, D., and Jones, F. H.. Frequency and diagnosis of hiatal hernia, Ann Surg 111.971, 1940.
20 Harrington, S. W.. Diagnosis and treatment of various types of diaphragmatic hernia, Am. J. Surg. 50:381, 1940.
21. Harrington, S. W.: Roentgenologic considerations in the diagnosis and treatment of diaphragmatic hernia, Am. J. Roentgenol 49.185, 1943.
22. Harrington, S. W.: Various types of diaphragmatic hernia treated surgically, Surg, Gynec & Obst 86 735, 1948
23 Harrington, S. W.: Traumatic diaphragmatic hernia, S. Clin North America 30.961, 1950.
24 Hildanus, F.. Cited by Buckstein (12).
25 Johnstone, A S.: Diagnosis of early gastric herniation at the esophageal hiatus, J Fac. Radiologists 3 52, 1951
26 Johnstone, A. S : The radiology of hiatus hernia, Proc. Roy. Soc Med 45 286, 1952
27. Jones, C. M : Hiatus esophageal hernia, New England J. Med 225·463, 1941
28 Keith, A The nature of the mammalian diaphragm and pleural cavities, J Anat. & Physiol 39 243, 1904–5
29 Kirklin, B. R, and Hodgson, J R Roentgenologic characteristics of diaphragmatic hernia, Am J Roentgenol. 58 77, 1947.
30 Lendrum, F C Anatomic features of the cardiac orifice of the stomach, Arch Int Med 59 474, 1937.
31 Master, A M, and Jaffe, H L. Coronary insufficiency and myocardial necrosis due to acute hemorrhage, J. Mt Sinai Hosp 7 26, 1940
32 Master, A. M, *et al* · Differential diagnosis of hiatus hernia and coronary artery disease, Arch Surg 58 428, 1949
33. Morgagni, G. B. *The Seats and Causes of Diseases Investigated by Anatomy* (London A Millar & T Cadell, 1769), Vol 3, p 210
34 Nuzum, F R Hernia of esophageal hiatus. Its relationship to angina pectoris, Am Heart J 33 724, 1947
34a Öhnell, H. Hernia diaphragmatica hiatus oesophagei vom intern klinischen Gesichtspunkt, Acta Radiol. 6 23, 1926.
35 Olsen, A. M, and Harrington, S W · Esophageal hiatal hernias of short esophagus type, etiologic and therapeutic considerations, J Thorac. Surg 17.189, 1948
36 Pack, G T, Butler, F S., and Katz, E The development of cancer in dystopic and ectopic thoracic stomachs, J Thorac Surg 23·593, 1952
37. Palmer, E D An attempt to localize the normal esophagogastric junction, Radiology 60 825, 1953
38. Paré, A. *Les Oeuvres d'Ambroise Paré* (Lyon 1664), Vol 2, p 250
39 Petit, J L *Traité des maladies Chirurgicals* (Paris· P F Didot, leJeune 1790), Vol 2, p 229.
39a Pickard Cited by Johnstone (25)
40. Redish, M H, and Kertzner, B Peptic ulcer of the esophagus, 18 579, 1951

The Determination of Insulin in Blood

A. F. WILLEBRANDS AND J GROEN

From the Second Medical Service, Wilhelmina-Gasthuis, Amsterdam, The Netherlands

MODERN ENDOCRINOLOGY is developing by leaps and bounds The demonstration by pioneer physiologists that the effects of removing certain glands could be neutralized by implanting the removed organs elsewhere in the body was a big step forward. In the following decades, specific clinical syndromes were found to be related to increased or decreased function of glands of internal secretion The next step was the use of crude endocrine extracts as replacement therapy for various conditions caused by endocrine hypofunction. The purification of these extracts followed logically. Methods for determining the levels of hormones and their metabolic products in blood and urine have only recently been perfected. Some of these are gradually coming into use in clinical investigations. The determination of the blood level of insulin has remained a problem which has long resisted the acumen of many investigators (47).

A reliable method for determining the insulin concentration in the blood is of obvious importance for the experimental physiologist and pathologist as well as for the clinician Such a method would make possible a much more precise study of the function of the islets of Langerhans, and would yield an insight into the factors influencing insulin production by the islet cells. The nature of both experimental and human diabetes might be elucidated if the insulin content of the blood in the pancreatic vein could be determined. Furthermore, as-

of animal weight, a rough linear relationship was demonstrable between the dose of insulin administered and the fall in blood sugar.

Gellhorn and co-workers (16) demonstrated the presence of insulin in normal human blood by means of such HA rats An hour after intraperitoneal injection of 4 ml. of blood taken 2 5 hours after lunch there was a significant fall in the blood sugar of the HA rats who had been given the injection On the basis of the extent of the drop in blood sugar, the insulin content of the injected blood was estimated to be about 2×10^{-4} units per milliliter. The effect did not depend on nervous stimulation of the pancreas, for it was also seen after sectioning of the vagus nerves Injection of the same blood into normal rats produced no observable effect

In 1947, Anderson and associates (2) used adrenodemedullated, alloxan diabetic, hypophysectomized rats (ADH rats) for the detection of insulin in rat's blood. In these animals the islet cells had been destroyed with alloxan to avoid interference by endogenously secreted insulin. The change in blood sugar was measured over a 30 minute period after the intravenous injection (under light nembutal anesthesia) of the material to be tested for the presence of insulin The animals were given a glucose meal 20 minutes prior to the injection, in order to produce a rising blood sugar base line and thus prevent a possibly fatal hypoglycemia during the test The rats were kept in a chamber at a temperature of 37 C. during the test. A significant decrease of the blood sugar developed after the injection of minimal amounts of insulin—as low as $0\,125 \times 10^{-3}$ units per rat A linear relationship was established between the changes in blood sugar and the logarithm of the insulin dose in the range of $0.1–5 \times 10^{-3}$ units per rat.

This method of estimating small amounts of insulin showed its usefulness in perfusion experiments of the isolated rat pancreas (1). It was demonstrated that the blood leaving the gland through the pancreatic vein produced no fall in blood sugar when injected into the ADH rat. But when additional glucose was added to the perfusion solution, the blood sugar of the ADH rat dropped significantly, an indication of increased insulin secretion by the islet cells in response to the high sugar content of the blood of the pancreatic artery The injection of 1 to 2 ml of plasma of normal rats fasted for 17 hours produced no demonstrable effect Human blood was not tested by these workers

says of the insulin concentration in the blood would help to establish the relative importance in human diabetes of hypoinsulinism and of hypersecretion of other hormones with a hyperglycemic action, such as glucagon. The technical aspects of measuring the insulin content of the blood have now been more thoroughly investigated, and at least two methods offer definite promise.

METHODS OF INSULIN ASSAY

In Vivo Method

This method makes use of the hypoglycemic effect of insulin. It was first tried in 1930 by Brugsch and Horsters (13), who attempted to measure the insulin content of the blood of untreated men and animals by injecting it into normal mice. There was no observable fall in blood sugar of the mice. They therefore concluded that the test animals were not sensitive enough It has now become apparent that the concentration of the hormone must exceed a level of 5×10^{-2} units per milliliter of blood before a positive result can be expected in an experiment such as devised by Brugsch and Horsters.

At about the same time several investigators demonstrated that animals could be rendered more sensitive to insulin by extirpation of certain glands: (*1*) Houssay and Magenta (23) found that hypophysectomy markedly increased the sensitivity of dogs to insulin. (2) Cannon and co-workers (15) demonstrated that cats, in response to insulin-induced hypoglycemia, secreted adrenalin, which releases sugar from the liver and thus counteracts the effect of hypoglycemia. Adrenodemedullated rats do not have such a reaction and are therefore more sensitive to insulin (*3*) Hemmingsen and associates (22) found that adrenalectomized rats and mice had an increased sensitivity to injection of insulin. 2×10^{-3} units of insulin produced a measurable fall in blood sugar in such mice

In 1941 Gellhorn *et al* (*16*) reported that the combination of demedullation of the adrenals and hypophysectomy increased the rat's sensitivity to insulin more than might be expected from either procedure alone In demedullated or hypophysectomized rats, a drop in blood sugar occurred with 5×10^{-3} units of insulin per *100* Gm. of rat; in hypophysectomized, adrenodemedullated rats (*HA* rats), even 3×10^{-4} units of insulin per 100 Gm. of rat caused a significant drop in blood sugar In the range of 3×10^{-4} to 10^{-3} units per *100* Gm

ported by Anderson *et al.*, was found to be valid also for the dose range of 5×10^{-5} to 5×10^{-4} units of insulin per rat. By means of this technic, Bornstein (5) was able to demonstrate the presence of insulin in human blood plasma. In the fasting state he found a concentration of 10^{-4} units per milliliter; a few hours after oral ingestion of glucose, the plasma insulin concentration was 2 to 3 times higher.

Yenerman *et al.* (51) have recently reported a demonstrable effect of 10^{-5} units of insulin and less in alloxan diabetic, hypophysectomized mice. The insulin was injected intraperitoneally 90 minutes after administration of 300 mg. of dextrin by stomach tube. The blood sugar level was assayed 1 hour later, and the fall in blood sugar was the measure of response. In the range between 10^{-4} and 10^{-5} units of insulin, the relationship of the response to the logarithm of the dose was linear, with a slope of 204 mg. per cent per tenfold dilution.

For the assay of blood samples with unknown insulin content, 8 animals were used, 2 at each of 2 dose levels for both the standard and the unknown. The authors do not give the results of such assays, but apparently believe that the mouse test has definite advantages over the ADHA rat test.

In Vitro Method

This method makes use of isolated rat diaphragm tissue Takane (42), about 25 years ago, was the first to use this method for *in vitro* study of metabolic processes. The advantages of this muscle preparation are that (*1*) the tissue can be easily excised without excessive injury to the muscle, and (*2*) it provides, because it is thin and flat, an extensive surface for contact with the incubation medium.

Using this muscle preparation, Gemmill (18–20) demonstrated the effect of insulin on carbohydrate metabolism *in vitro* When the isolated rat diaphragm was incubated in a glucose buffer solution, glucose was utilized and glycogen was deposited in the muscle. The addition of insulin to the incubation medium markedly stimulated the utilization of glucose and the formation of glycogen Several investigators (27, 37, 39, 49), some of whom have studied more closely the quantitative aspects of this effect of insulin on the rat's diaphragm *in vitro*, subsequently confirmed this fundamental observation. Table 1, which summarizes the results of these studies, shows that there are wide variations in the sensitivity of the rat diaphragm to insulin.

These differences in sensitivity are due to a number of factors,

In 1949, Bornstein (6), using a modification of the above technic, obtained significant effects with insulin doses as low as 5×10^{-5} units per rat. He prepared alloxan diabetic, hypophysectomized, adrenalectomized rats (ADHA rats) as follows:

Adult male rats were made diabetic by an intravenous injection of alloxan (50 mg./Kg.), 36 hours later, when the animals exhibited severe glucosuria and ketonuria, they were given protamine zinc insulin for a few days. The insulin was then withdrawn for 2 days and hypophysectomy was performed. The adrenals were removed after the hypophysectomy wounds had healed.

These ADHA rats are difficult to prepare and maintain. The mortality of the complicated preparatory procedure is 33 per cent. The rats are especially sensitive to starvation and cold, but when they are kept under good conditions at temperatures of 22 to 26 C and given unlimited food, they may live up to 8 months and can be used several times.

Bornstein's assay technic differs from that of Anderson and associates (1, 2) in several respects: (*1*) Insulin or the material to be tested for insulin content is administered subcutaneously instead of intravenously; anesthesia, which influences carbohydrate metabolism, is thereby avoided. (*2*) The effect being measured, that is, the change in blood sugar concentration in 1 hour, although slower to develop, is more pronounced (*3*) The test is performed during a period when the blood sugar base line is flat The conditions necessary to obtain such a stable blood sugar base line were carefully investigated in preliminary tests, diet and temperature appeared to have a considerable influence Control assays were performed with 1 ml of physiologic saline instead of insulin solution or plasma; no change in blood sugar should occur with the injection of saline

The test itself is performed as follows (7) The animals, kept at 27 C, are fed bread soaked in milk (the diet found most suitable) during a 90 minute period. Food is then withheld for another 90 minutes, after which 1 ml. of heparinized plasma or insulin solution is injected, 1 hour later the fall in blood sugar is determined.

With this procedure, the blood sugar base line was found to remain level in control animals from the third to the fifth hour after the start of the test

The direct relationship between the logarithm of the amount of insulin injected and the drop in blood sugar concentration, as re-

was invariably greater when hemidiaphragms were used than when the diaphragms had been divided into 4 or 8 parts.

6 *Technic used.*—Most investigators use the single diaphragm technic, in which the diaphragm of an animal is divided into 2 or more approximately equal parts. Each part is put into a separate flask containing the medium to be tested. Only 1 animal is used for each experiment, with 1 section of diaphragm serving as control for the other section(s) of the same diaphragm. Since the diaphragms from different rats differ widely in the magnitude of their glucose utilization, glycogen formation, and the insulin effect on these proc-

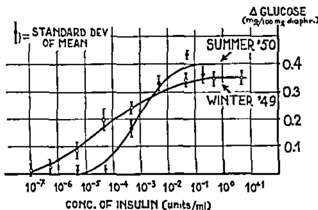


Fig. 1.—Variability of concentration curve of quantitative effect of insulin on glucose utilization of isolated rat diaphragm (21).

esses, the experiment must be repeated many times to obtain statistically valid results

A modified technic (48), which is described in detail below, largely eliminates the factor of animal variation and reduces the number of experiments Each of the diaphragms of 8 rats are divided into 4 pieces The tissues are pooled and placed in 4 flasks in such a manner that each flask contains 8 quarter diaphragms, 1 from each of the rats. Thus, although every experiment requires 8 animals, the number of experiments is reduced. This pooled diaphragm technic seems to be better than the single diaphragm method (37).

However, some as yet unknown factors must also influence the magnitude of the insulin effect, for the sensitivity of the diaphragms varies even when all conditions with respect to the above-mentioned factors are standardized (21, 48). Figure 1, for example, gives two

some well known, others still obscure. The following factors are known to influence the effect of insulin on the rat diaphragm:

1. *Mineral composition of the incubation medium.*—Stadie and Zapp (39) report that the magnitude of the insulin effect depends on the concentration of potassium and magnesium, the effect being greatest at concentration zero and very small when the potassium or magnesium concentration is raised to 75 per cent of the total cation concentration. Obviously, this is an entirely unphysiologic proportion of these electrolytes. Between 6.3 and 7.6, the pH of the incubation medium has little influence.

2. *Glucose concentration of the medium.*—The same authors showed that utilization of glucose and formation of glycogen increases

TABLE 1.—SENSITIVITY OF RAT DIAPHRAGM TO INSULIN

REFERENCE	SMALLEST DETECTABLY CONC. OF INSULIN, U/ML	FUNCTION AFFECTED	TECHNIC
Stadie & Zapp (39)	$1-3 \times 10^{-3}$ (no effect with 5×10^{-4})	Glycogen formation	Single diaphragm method, ½ diaph.
Krahl & Park (27)	10^{-4} (with 10^{-5}, insignificant effect)	Glucose utilization	Single diaphragm method, ¼ diaph.
Perlmutter *et al* (37)	$\pm 5 \times 10^{-3}$	Glycogen synthesis	Single diaphragm method, ¼ diaph.
Willebrands *et al.* (49)	$\pm 10^{-4}$	Glucose utilization	Pooled diaphragm method, ¼ diaph

when the glucose concentration is raised from 0.1 to 1 per cent, and that the effect of insulin on both processes also increases.

3. *Fasting.*—On the other hand, Perlmutter and Greep (36) found that the glucose utilization, as well as the effect of insulin upon this utilization, were greater when the rats were fasted for 4 days than for 24 hours, as is the common practice of most investigators. Willebrands *et al* (50) came to similar conclusions.

4. *Age.*—Young rats of 80 to 100 Gm. weight give far better results than adult rats (24). This is probably connected with the fact that the relationship between mass and surface is more advantageous in the diaphragms of young rats. The same factor may be the reason why the relatively thick diaphragms of adult mice were found to be unsuitable for this test.

5. *Trauma.*—Injury to the diaphragm tissue during excision and subsequent handling inhibits the insulin effect (24, 48), the latter

was invariably greater when hemidiaphragms were used than when the diaphragms had been divided into 4 or 8 parts.

6. *Technic used.*—Most investigators use the single diaphragm technic, in which the diaphragm of an animal is divided into 2 or more approximately equal parts. Each part is put into a separate flask containing the medium to be tested. Only 1 animal is used for each experiment, with 1 section of diaphragm serving as control for the other section(s) of the same diaphragm Since the diaphragms from different rats differ widely in the magnitude of their glucose utilization, glycogen formation, and the insulin effect on these processes, the experiment must be repeated many times to obtain statistically valid results

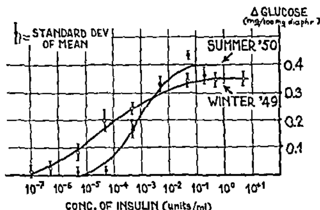


FIG. 1—Variability of concentration curve of quantitative effect of insulin on glucose utilization of isolated rat diaphragm (21).

A modified technic (48), which is described in detail below, largely eliminates the factor of animal variation and reduces the number of experiments Each of the diaphragms of 8 rats are divided into 4 pieces The tissues are pooled and placed in 4 flasks in such a manner that each flask contains 8 quarter diaphragms, 1 from each of the rats Thus, although every experiment requires 8 animals, the number of experiments is reduced. This pooled diaphragm technic seems to be better than the single diaphragm method (37)

However, some as yet unknown factors must also influence the magnitude of the insulin effect, for the sensitivity of the diaphragms varies even when all conditions with respect to the above-mentioned factors are standardized (21, 48). Figure 1, for example, gives two

some well known, others still obscure. The following factors are known to influence the effect of insulin on the rat diaphragm:

1. *Mineral composition of the incubation medium.*—Stadie and Zapp (39) report that the magnitude of the insulin effect depends on the concentration of potassium and magnesium, the effect being greatest at concentration zero and very small when the potassium or magnesium concentration is raised to 75 per cent of the total cation concentration. Obviously, this is an entirely unphysiologic proportion of these electrolytes. Between 6 3 and 7.6, the pH of the incubation medium has little influence.

2. *Glucose concentration of the medium.*—The same authors showed that utilization of glucose and formation of glycogen increases

TABLE 1 —SENSITIVITY OF RAT DIAPHRAGM TO INSULIN

REFERENCE	SMALLEST DETECTABLE CONC. OF INSULIN, U./ML.	FUNCTION AFFECTED	TECHNIC
Stadie & Zapp (39)	$1\text{–}3 \times 10^{-3}$ (no effect with 5×10^{-4})	Glycogen formation	Single diaphragm method, ½ diaph.
Krahl & Park (27)	10^{-4} (with 10^{-5}, insignificant effect)	Glucose utilization	Single diaphragm method, ¼ diaph.
Perlmutter *et al* (37)	$\pm 5 \times 10^{-5}$	Glycogen synthesis	Single diaphragm method, ¼ diaph.
Willebrands *et al* (49)	$\pm 10^{-5}$	Glucose utilization	Pooled diaphragm method, ¼ diaph

when the glucose concentration is raised from 0 1 to 1 per cent, and that the effect of insulin on both processes also increases.

3 *Fasting.*—On the other hand, Perlmutter and Greep (36) found that the glucose utilization, as well as the effect of insulin upon this utilization, were greater when the rats were fasted for 4 days than for 24 hours, as is the common practice of most investigators. Willebrands *et al.* (50) came to similar conclusions

4. *Age —Young rats of 80 to 100 Gm. weight give far better results* than adult rats (24) This is probably connected with the fact that the relationship between mass and surface is more advantageous in the diaphragms of young rats The same factor may be the reason why the relatively thick diaphragms of adult mice were found to be unsuitable for this test

5. *Trauma.*—Injury to the diaphragm tissue during excision and subsequent handling inhibits the insulin effect (24, 48); the latter

be due to the presence in the serum of substances other than insulin was ruled out by the following observations:

1. Human serum obtained during diabetic coma, or serum of dogs in diabetic coma after pancreatectomy, did not increase significantly the glucose utilization of the diaphragm tissue

2 Serum of diabetic patients under insulin treatment had a stimulating effect of the same magnitude as that of normal human serum

3 The stimulating effect of the serum of a depancreatized dog

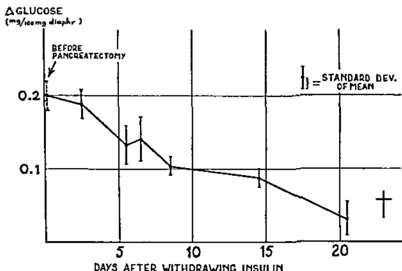


Fig 2—Diminution of "insulin effect" of serum of a depancreatized dog, tested on isolated rat diaphragm (21)

maintained with insulin gradually decreased after withdrawal of the insulin. Shortly before the dog's death in coma, the effect of the serum was almost nil (Fig 2)

4. Glucose utilization was significantly greater when diaphragms were incubated in buffer and serum obtained from a patient after recovery from coma than in buffer and serum from the same patient before treatment of the coma

5 The possibility that the difference between normal serum and serum taken during diabetic coma could be due to inhibition of glucose utilization by some substance present in the latter seems un-

concentration action curves established at different times, showing that the response of the diaphragm tissue to the same amounts of insulin, especially in the region of the lower insulin concentrations, differs markedly. A seasonal factor is apparently not the reason for the variation, but the exact cause has not yet been established.

The many known and unknown factors influencing the insulin sensitivity of diaphragm muscle preparations must be responsible for the discrepancies in the smallest amount of insulin which could be detected by different investigators.

Although it has been proved that the rat diaphragm test reveals the presence of minute amounts of insulin (10^{-3} units or less per milliliter of incubation medium), there are only a few reports on the use of the test to detect insulin in blood. The extremely low insulin concentration in the blood, together with the great variability in sensitivity of the rat diaphragm, must have discouraged many workers from using this method for determining the insulin level in serum.

One study of the *in vitro* synthesis of glycogen in the diaphragms of normal and alloxan diabetic rats records an experiment showing that the diaphragm of a normal rat synthesized more glycogen when incubated in serum than in a buffer of the composition of "extra-cellular solution" (43).

Perlmutter and Greep (35), too, found that human blood serum has a stimulating effect on the glycogen synthesis of the isolated rat diaphragm In later studies, the same workers were unable to detect the presence of insulin in normal human serum; furthermore, with the rat diaphragm test, they did not obtain an insulin effect with blood serum taken after intravenous injection of 25 Gm of glucose. However, an increase in the serum insulin concentration was detected 5 minutes after the intravenous injection of 20 units of insulin

Other workers, however, using the pooled diaphragm technic, were able to demonstrate regularly that insulin is present in normal human and normal dog's blood (21, 25, 48) The insulin concentration in the serum was estimated to vary between 5×10^{-5} and 5×10^{-4} units per milliliter When the pooled hemidiaphragms of 4 rats were incubated in buffer alone and the other hemidiaphragms of the same 4 rats in buffer and 10 to 20 per cent serum of normal man or dog, the glucose utilization and glycogen synthesis were invariably greater in the buffer–serum mixture than in the buffer alone. The differences were statistically significant.

The possibility that this stimulating effect of normal serum might

then be calculated from the influence the addition of serum exerts upon glucose utilization or glycogen synthesis by the rat diaphragm preparation. As is evident from the curve shown in Figure 3, this method yields a fairly rough estimate of the insulin concentration in serum. For example, an increase in insulin concentration from 10^{-4} to 2×10^{-4} units per milliliter would not cause a significant increase in glucose utilization; a difference between 10^{-4} and 5×10^{-4} units, however, would cause a significant difference in effect. Furthermore, as emphasized above, the sensitivity of the diaphragm tissue may vary from experiment to experiment as a result of unknown factors. This difficulty is overcome by assaying, in every test, two known concentrations of insulin concurrently with the unknown serum.

The actual test, as described by Groen *et al.* (21) is performed as follows:

Eight young rats, weighing 80 to 100 Gm., are decapitated after having been fasted for 24 hours. The diaphragms are taken out quickly, with the greatest care to avoid trauma, and quartered. The 32 quarters are collected into 4 beakers containing ice-cold buffer solution (without glucose); each beaker should contain a quarter diaphragm from each of the 8 rats (4 from the left side, 4 from the right). The diaphragms are removed from the beakers, blotted on filter paper, and placed in 4 Erlenmeyer flasks of 25 ml capacity. Flask 1 contains 2 ml of a buffer solution with a mineral composition closely resembling blood plasma, flask 2, the same buffer solution mixed with 10 to 20 per cent of the serum to be investigated, flasks 3 and 4, buffer solution with pure insulin in known concentrations (usually 10^{-4} and 10^{-3} units per milliliter). All incubation mediums contain 200 mg per cent of glucose, they are equilibrated with a gas mixture of 93 per cent oxygen and 7 per cent carbon dioxide to have a pH of about 7.4. The flasks are cooled in ice water during the transfer. Following renewed equilibration with the gas mixture, the flasks are incubated for 90 minutes at 37 C., being shaken at 120 strokes per minute during the incubation. The flasks are then removed from the incubator and cooled in ice water. The muscle tissue is removed from the flasks, dried between filter paper, and weighed. The glucose concentration in the medium in each flask is determined, the difference in glucose utilization per 100 mg of diaphragm tissue between flasks 2, 3, and 4, and flask 1 (control) being used for the calculation. The whole experiment is run six times (a total of 48 rats) in order to make statistical calculation possible. In addition, the amount of glycogen synthesized in the diaphragms is determined. This figure can also be used for calculating the insulin content of the serum, but the effects on glycogen synthesis are invariably smaller than those on glucose utilization.

likely, because: (*a*) the glucose utilization in diluted coma serum did not differ significantly from that in buffer without serum; (*b*) the effect of known small concentrations of insulin (10^{-4} units per milliliter) on the rat diaphragm was not diminished when coma serum was added to the incubation medium, (*c*) the addition of acetone and beta-hydroxybutyric acid to normal serum did not influence the effect of this serum.

6. Just as the effect of pure insulin disappears under the influence of cysteine or glutathione, the stimulating effect of normal serum

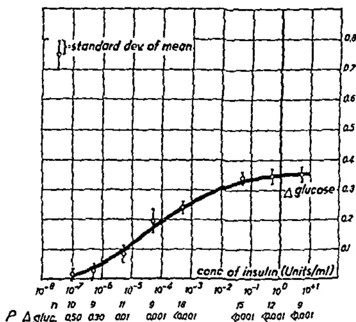


FIG 3.—Concentration of action curve showing increase in glucose utilization of isolated rat diaphragm by different concentrations of insulin in the medium (21)

diminishes considerably or disappears when the serum is treated with these two substances

7. Like the effect of pure insulin, the stimulating effect of serum in the rat diaphragm test is greater when hemidiaphragms are used than when diaphragms are divided into 4 or 8 parts.

A standard curve is obtained by means of a series of experiments with different concentrations of pure insulin (Fig. 3). With the help of this standard curve, the concentration of insulin in the serum can

rat serum (4) Comparison of the effect of normal serum with that of the serum of diabetic rats previously hypophysectomized or adrenalectomized disclosed no difference in glucose utilization Bornstein therefore assumes that the blood of alloxan diabetic rats contains an insulin antagonist which is absent after hypophysectomy or adrenalectomy.

There are no exact data on the influence of steroids on the insulin sensitivity of the ADHA rats Bornstein and Lawrence (7) state that cortisone and ACTH had no effect, but give no details, the effect of other steroids was not studied.

The possibility that in the rat diaphragm test, also, hormones other than insulin may exert an interfering effect must at least be considered It has been shown (28, 44, 45) that the effect of insulin on glycogen synthesis and glucose uptake of normal rat diaphragms was greatly diminished when certain steroid compounds were added to the incubation medium Desoxycorticosterone, progesterone, and to a smaller extent, corticosterone, 17-hydroxycorticosterone, and testosterone were found to be active in this respect. For these experiments, an insulin concentration of 10^{-2} units per milliliter and a steroid concentration of 10 to 100 gamma per milliliter of incubation medium were used Other investigators (3), using radioactive (C^{14}) glucose, confirmed these findings at least so far as the effect of steroids on glycogen synthesis is concerned In the experiments with C^{14}, glucose utilization was not affected by the steroids. It is noteworthy that this effect on glycogen synthesis *in vitro* did not parallel the glucocorticoid activity of the substances Other workers found that· (*1*) 50 gamma of 17-hydroxycorticosterone or 11-desoxycorticosterone per milliliter of incubation medium diminished the glycogen synthesis in the rat diaphragm, as compared to controls (40), (*2*) 3 4 to 7 2 gamma of cortisone per 100 mg of diaphragm tissue decreased glucose utilization in the rat diaphragm (14), (*3*) 25 gamma of cortisone per milliliter of incubation medium slightly diminished glucose utilization in the diaphragm, but left unchanged the effect of 5×10^{-4} units of insulin per milliliter (24).

In almost all the experiments with steroids, the concentration of these substances added to the medium was higher, and often much higher, than might be expected in tests with diluted serum. The normal concentration of 17-hydroxysteroids in normal serum, for example, ranges between 5 and 30 gamma per 100 milliliters, according to Nelson and Samuels (32, 33), the highest figure they found was in a case

DISCUSSION

As may be gathered from the foregoing, it is fairly difficult to estimate the minute quantities of insulin present in blood. Only recently have two methods been evolved for a more or less quantitative estimation, although both are still imperfect. Anderson's and Bornstein's *in vivo* methods seem sensitive and not too difficult to perform, but they require specially prepared rats which must be carefully kept and controlled. On the other hand, the rat diaphragm test, though equally sensitive, must take into account the large variation in response of this tissue, the method, therefore, requires scrupulous standardization and multiple control experiments.

The possible influence of hormones other than insulin present in serum on the results obtained with both methods must also be considered According to Bornstein (4), his ADHA method does not disclose the absolute amount of insulin present in the test material (heparinized plasma) but only "the excess of insulin over its antagonists absorbed in unit time." Even when no insulin is found, it is possible that insulin is present in the plasma but is neutralized by some inhibitor or antagonist Bornstein cites several reasons for this belief. (*1*) After injection of ADHA rats with plasma from diabetic patients in which no insulin could be detected, the sensitivity of the ADHA rats to insulin was diminished (4, 9); this might be explained by the assumption that the serum of these diabetic patients contained some inhibitor which prevented insulin from acting. (*2*) Portal blood sometimes even peripheral blood from ADHA rats or from intact cats, exerts a hyperglycemic effect on recipient ADHA rats when the donor animals have been pretreated with growth hormone to induce diabetes (12). Such a hyperglycemic effect was not found when the growth hormone was administered directly to the recipient rats, although a delayed transient rise occurred in some animals Possibly the portal blood of the donor animals contained the hyperglycemic factor (glucagon) liberated by the alpha cells of the pancreatic islets under the influence of the growth hormone. Since the peripheral blood of the animals receiving growth hormone usually exerts no hyperglycemic action, the hyperglycemic substance, unlike insulin, does not seem to reach the peripheral veins in appreciable amounts (*3*) The glucose utilization of normal diaphragm tissue, when incubated in a medium containing serum of alloxan diabetic rats, was lower than when incubated in a medium containing the same amount of normal

rat serum (4). Comparison of the effect of normal serum with that of the serum of diabetic rats previously hypophysectomized or adrenalectomized disclosed *no difference in glucose utilization* Bornstein therefore assumes that the blood of alloxan diabetic rats contains an insulin antagonist which is absent after hypophysectomy or adrenalectomy.

There are no exact data on the influence of steroids on the insulin sensitivity of the ADHA rats Bornstein and Lawrence (7) state that *cortisone and ACTH had no effect, but give no details, the effect of* other steroids was not studied.

The possibility that in the rat diaphragm test, also, hormones other than insulin may exert an interfering effect must at least be considered. It has been shown (28, 44, 45) that the effect of insulin on glycogen synthesis and glucose uptake of normal rat diaphragms was greatly diminished when certain steroid compounds were added to the incubation medium Desoxycorticosterone, progesterone, and to a smaller extent, corticosterone, 17-hydroxycorticosterone, and testosterone were found to be active in this respect. For these experiments, an insulin concentration of 10^{-2} units per milliliter and a steroid concentration of 10 to 100 gamma per milliliter of incubation medium were used Other investigators (3), using radioactive (C^{14}) glucose, confirmed these findings at least so far as the effect of steroids on glycogen synthesis is concerned. In the experiments with C^{14}, glucose utilization was not affected by the steroids It is noteworthy that this effect on glycogen synthesis *in vitro* did not parallel the glucocorticoid activity of the substances Other workers found that: (*1*) 50 gamma of 17-hydroxycorticosterone or 11-desoxycorticosterone per milliliter of incubation medium diminished the glycogen synthesis in the rat diaphragm, as compared to controls (40), (*2*) 3 4 to 7 2 gamma of cortisone per 100 mg of diaphragm tissue decreased glucose utilization in the rat diaphragm (14), (*3*) 25 gamma of cortisone per milliliter of incubation medium slightly diminished glucose utilization in the diaphragm, but left unchanged the effect of 5×10^{-4} units of insulin per milliliter (24).

In almost all the experiments with steroids, the concentration of these substances added to the medium was higher, and often much higher, than might be expected in tests with diluted serum The normal concentration of 17-hydroxysteroids in normal serum, for example, ranges between 5 and 30 gamma per 100 milliliters, according to Nelson and Samuels (32, 33), the highest figure they found was in a case

of Cushing's disease—100 gamma per 100 ml. of serum. With a 20 per cent serum dilution, such as used for the rat diaphragm test, the concentration of this steroid in the incubation medium would normally be 0.01 to 0.06 gamma per milliliter, and in the case of Cushing's disease it would have been 0.2 gamma per milliliter or less than a tenth of the lowest concentration found to have an effect in the rat diaphragm test. It therefore seems improbable that the glucose metabolism of the isolated rat diaphragm would be affected by the amounts of glucocorticoid hormones normally or pathologically present in human blood.

Reports in the literature on a possible influence of pituitary hormones in the rat diaphragm test are somewhat confusing. Many investigators have worked with diaphragms of hypophysectomized rats or of rats treated with anterior pituitary extract or growth hormone (8, 10, 27, 29, 30, 35, 36). But the influence on glucose uptake or glycogen synthesis in the rat's diaphragm when anterior pituitary extract or growth hormone are added to the incubation medium or on the effect of low insulin concentrations thereupon has scarcely been studied. As normal rat diaphragms are used in the rat diaphragm test, and the serum is the variable to be tested, only studies on this aspect of the problem will be discussed here.

Stadie *et al.* (41) found that the effect of insulin upon glycogen synthesis of normal diaphragm tissue is diminished when the incubation medium contains crude anterior pituitary extract, purified growth hormone had no effect. Park *et al* (34), however, reported that the addition of either crude or purified growth hormone in a concentration of 100 gamma per cubic centimeter of incubation medium had no perceptible effect on glucose uptake or on the effect of insulin (4 gamma per milliliter = 0.1 unit per milliliter) on this uptake

On the whole, such findings would seem to indicate that hormones other than insulin may play a role in the tests with ADHA rats or with rat diaphragms So far, however, the quantitative aspects of the influence of these hormones have been inadequately studied, especially in dose ranges to be expected in normal or pathologic serums. It is possible that in some of the ADHA tests the blood contained a substance masking the influence of insulin, which might have led to false interpretations of the results obtained. This would be particularly true of the blood of patients with increased levels of pituitary hormones and perhaps also of the hyperglycemic factor It seems less likely that

such interference by substances other than insulin operates in the rat diaphragm test.

RESULTS

NORMAL VALUES.—Table 2 summarizes the values for blood insulin under normal conditions in man, dog, and rat, as determined by a number of workers by various methods. All the figures substantiate

TABLE 2.—INSULIN CONTENT OF BLOOD, PLASMA, OR SERUM OF NORMAL MAN OR ANIMAL

REFERENCE	METHOD*	MATERIAL TESTED†	INSULIN CONC., U./ML.	SPECIES TESTED
Brugsch & Horsters (13)	h.e. in normal mice	W	$<5 \times 10^{-2}$	Man, dog, rabbit
Gellhorn *et al.* (16)	h.e. in HA rats	W	$\pm 2 \times 10^{-4}$	Man, 2 hr. after lunch
Gellhorn *et al.* (17)	h.e. in HA rats	W	$\pm 10^{-4}$	Dog fasted 24 hr.
Gellhorn *et al.* (17)	h.e. in HA rats	W	$\pm 10^{-4}$	Rabbit
Anderson *et al.* (2)	h.e. in ADH rats	P	$<10^{-4}$	Rat fasted 17 hr.
Perlmutter *et al.* (37)	RD test	P	$<5 \times 10^{-3}$	Man
Bornstein *et al.* (5)	h.e. in ADHA rats	P	1×10^{-4}	Man, fasting
Bornstein *et al.* (5)	h.e. in ADHA rats	P	$2.4–4.0 \times 10^{-4}$	Man, 2 hr. after 50 Gm. glucose orally
Groen *et al.* (21)	RD test	S	$5 \times 10^{-5}–5 \times 10^{-4}$	Man, dog

* h.e., hypoglycemic effect; HA, hypophysectomized, adrenodemedullated; ADH, alloxan diabetic, adrenodemedullated, hypophysectomized; ADHA, alloxan diabetic, adrenalectomized hypophysectomized; RD, rat diaphragm.
† W, whole blood; P, plasma; S, serum.

the assumption that the insulin concentration in normal human serum varies around 10^{-4} units per milliliter. According to Bornstein (5), the concentration in the fasting state is 10^{-4} units per milliliter, and increases during a glucose tolerance test to about 3×10^{-4} units.

VALUES IN DIABETES MELLITUS.—No insulin could be demonstrated with the rat diaphragm test in serum taken from patients in severe diabetic coma, and it was therefore concluded that the insulin content of such serum must be below 10^{-5} units per milliliter (21, 25). Normal or slightly elevated values (10^{-4} to 10^{-3} units) were found in the

serum of the same patients after recovery from diabetic coma and in that of patients under insulin treatment, even when the serum was taken 18 hours after the last insulin injection.

The ADHA method was used to determine the plasma insulin content of 8 untreated cases (11), and later of 28 untreated cases (7), of diabetes. The serum was taken 2 hours after ingestion of 50 Gm. of glucose by mouth On the basis of the test results, the patients were divided into two groups: one with and one without a demonstrable amount of insulin in the blood. As a rule, the patients in the first group were elderly, obese women without signs of ketosis, and in most of them the diabetes could be controlled by diet alone The presence of insulin was regularly demonstrable in their blood plasma, the average value being 2.3×10^{-4} units per milliliter (± 70 per cent of the mean normal value).

The second group was made up of patients of various ages, all had lost weight, all had signs of ketosis, and all required insulin. No insulin could be detected in their blood plasma before treatment; after they were put on insulin, normal values were obtained (2 to 2.6×10^{-4} units per milliliter) Bornstein and co-workers offer several explanations for the negative results obtained in this group: (1) It is possible that no insulin or very little was secreted by the pancreas. (2) Insulin may not have been found because of its rapid destruction (Mirsky and Broh-Kahn [31] have demonstrated the existence of insulinase systems) Should this explanation be valid, the destruction must occur in the tissues and not in the plasma For when the plasma of these patients was incubated for 4 hours at 37 C with 0.2 units of insulin per milliliter, the hormone was not destroyed (11). (3) The plasma in question might have contained factors antagonistic to insulin, particularly if the pituitary was hyperactive

As has already been pointed out, our own results (21) differ from those of Bornstein and Lawrence (7). With the rat diaphragm test, no sharp distinction could be found between diabetic patients with and those without insulin in the blood, except during diabetic coma

HYPERINSULINISM —Only a small number of patients suffering from spontaneous hypoglycemic attacks have been examined for the insulin content of their blood.

Hypophysectomized, adrenodemedullated (HA) rats were used for testing the blood of 2 such patients, and an increased insulin content, as compared with that of normal human blood, was found (16). The serum of another patient was tested by means of alloxan diabetic,

hypophysectomized, adrenalectomized (ADHA) rats, a high insulin concentration must have been present in this serum, for the rats died of hypoglycemia (4). Neither report gives exact values for the insulin content of the serum tested.

Assay of serum for insulin content seems to offer a possibility for direct demonstration of true hyperinsulinemia and for a differential diagnosis of this condition from functional hypoglycemia. Using the rat diaphragm test, we examined the serum of 1 patient with functional hypoglycemia and found the insulin content to be within normal limits (about 10^{-4} units per milliliter). In a case of islet cell adenoma (diagnosis confirmed at operation), on the other hand, the insulin content was about 3×10^{-3} units per milliliter, a significantly higher value than that of normal serum. Three additional cases of islet cell adenoma have since been examined, the insulin content was extremely high in all three (6×10^{-3} to 8×10^{-3} units per milliliter). An islet cell tumor was removed on operation in all 3 patients, and the insulin content thereafter dropped to normal limits (50). A serum specimen of one of the patients obtained during an asymptomatic interval had a normal insulin content, (although the patient was fasting at the time). Thus it seems worth emphasizing that a single normal value for blood insulin is insufficient evidence for excluding the possibility of islet cell adenoma, repeated determinations on blood drawn during attacks are advisable before establishing the diagnosis definitely

INSULIN RESISTANCE —The blood plasma of several insulin-resistant patients has been examined, with contradictory results. (*1*) The ADHA test was used for 4 patients requiring up to 2,000 units of insulin per day, no insulin could be detected in the plasma taken 1 hour after injection of 300 units of insulin (7, 11) (*2*) The rat diaphragm test in a patient with this type of diabetes disclosed no insulin activity in the serum (50). (*3*) The serum of another insulin-resistant patient who required 300 to 400 units of insulin per day contained a substance inhibiting the action of insulin, as demonstrated by Stadie's dipping variant of the rat diaphragm test (38). (*4*) In a patient requiring 2,000 units of insulin per day, the rat diaphragm test revealed the serum insulin content to be about 5×10^{-3} units per milliliter just before intravenous administration of 600 units of insulin, and far higher 5 minutes after the administration (50)

The assumption that insulin resistance may be due to a variety of causes would explain the apparently contradictory results reported First, insulinase systems which destroy insulin may be present. Sec-

ond, inhibiting substances may prevent the action of insulin. Third. antibodies to insulin protein may precipitate the insulin and thus remove it from the circulation.

OTHER CONDITIONS.—Psychotic patients have been shown to have an increased insulin concentration in the blood during periods of excitement (16).

The serum of 2 patients with acromegaly produced a rise, instead of a fall, in the blood sugar of ADHA rats; these patients had hyperglycemia and glucosuria, but there were no signs of ketosis and insulin was not required (7).

In 1 case of lipoatrophic diabetes the serum contained insulin (7), exact figures were not given.

SUMMARY

Two methods—one *in vivo*, the other *in vitro*—are now available for the determination of the insulin content of blood. The *in vivo* method, and its modifications, makes use of animals whose hyperglycemic response has been standardized by the injection of alloxan and who have been rendered highly susceptible to minute amounts of insulin by adrenodemedullation (or adrenalectomy) and hypophysectomy.

The *in vitro* method is based on the observation that minute amounts of insulin increase the glucose utilization by the diaphragm tissue of young rats

By both methods the insulin content in normal human serum has been shown to be about 10^{-4} units per milliliter During diabetic coma and after pancreatectomy, no detectable amounts of insulin have been found in the blood, whereas the serum of patients with islet cell adenoma shows an increased insulin concentration.

REFERENCES

1. Anderson, E., and Long, J A The effect of hyperglycemia on insulin secretion as determined with the isolated rat pancreas in a perfusion apparatus, Endocrinology 40 92, 1947
2. Anderson, E , Lindner, E , and Sutton, V A sensitive method for the assay of insulin in blood, Am J Physiol 149 350, 1947
3. Bartlett, G R., Wick, A N , and MacKay, E M The influence of insulin and adrenal cortical compounds on the metabolism of radioactive C^{14}-glucose in the isolated rat diaphragm, J Biol Chem 178 1003, 1949
4. Bornstein, J The insulin content of blood plasma, Diabetes 2 23, 1953
5. Bornstein, J · Normal insulin concentration in man, Australian J Exper. Biol. & M Sc. 28 93, 1950

6 Bornstein, J.· A technique for the assay of small quantities of insulin using alloxan diabetic, hypophysectomized, adrenalectomized rats, Australian J. Exper. Biol & M. Sc. 28.87, 1950
7. Bornstein, J, and Lawrence, R D Plasma insulin in human diabetes mellitus, Brit M J 2.1541, 1951.
8. Bornstein, J, and Nelson, J F. Effect of hypophysectomy on glycogen deposition in isolated muscle, Nature, London 162 572, 1948
9 Bornstein, J, and Trewhella, P.. An anti-insulin factor in the plasma of diabetic patients, M J Australia 2.179, 1950, 1.119, 1951.
10 Bornstein, J, and Trewhella, P. Endocrine influences on glycogen deposition in the isolated rat's diaphragm, Australian J. Exper. Biol. & M Sc. 28. 573, 1950
11. Bornstein, J, and Trewhella, P. Plasma insulin levels in diabetes mellitus in man, Australian J Exper Biol. & M. Sc. 28 569, 1950
12 Bornstein, J, Reid, E, and Young, F. C· The hyperglycaemic action of blood from animals treated with growth hormone, Nature, London 168 903, 1951.
13 Brugsch, H., and Horsters, H.. Insulingehalt und Insulinverteilung im Blut, Arch exper Path u. Pharmakol. 148 295, 1938
14 Candela, J-L R, *et al* Influencia de la cortisona sobre el consumo de glucosa por el diafragma aislado de rata, Arch med exper 14 7, 1951.
15 Cannon, W B, McIver, M A, and Bliss, S W. Studies on the conditions of activity in endocrine glands 13 A sympathetic and adrenal mechanism for mobilizing sugar in hypoglycemia, Am J Physiol 69 46, 1924
16 Gellhorn, E, Feldman, J, and Allen, A.. Assay of insulin on hypophysectomized, adreno-demedullated, and hypophysectomized-adreno-demedullated rats, Endocrinology 29 137, 1941
17. Gellhorn, E, Feldman, J, and Allen, A Insulin concentration in the blood of normal and pancreatectomized dogs, Endocrinology 29 849, 1941
18 Gemmill, C. L. The effect of insulin on the glycogen content of isolated muscles, Bull Johns Hopkins Hosp 66 232, 1940.
19. Gemmill, C L.. The effects of glucose and of insulin on the metabolism of the isolated diaphragm of the rat, Bull Johns Hopkins Hosp 68 329, 1941
20 Gemmill, C L, and Hamman, L, Jr The effect of insulin on glycogen deposition and on glucose utilization by isolated muscles, Bull Johns Hopkins Hosp 68 50, 1941
21 Groen, J, *et al* Evidence for the presence of insulin in blood serum A method for an approximate determination of the insulin content of blood, J Clin Invest 31 97, 1952
22 Hemmingsen, A M, Nielsen, A, and Nielsen, A L The effect of insulin on adrenalectomized rats and mice, Acta med scandinav (supp) 90 105, 1938
23 Houssay, B A, and Magenta, M A Sensibilidad en los perros hipofisoprivos a la insulina, Rev med argent 37·389, 1924, abstr in Compt rend Soc biol 92 822, 1925
24 Kamminga, C E, Willebrands, A. F, and Groen, J Unpublished data.
25 Kamminga, C E, *et al* De invloed van insuline op de glycose- en kaliumhuishouding van het geisoleerde rattendiaphragma Een methode voor

het aantonen van insuline in bloedserum [Influence of insulin on dextrose and potassium metabolism of isolated diaphragm of rat Method for determination of insulin in blood serum], Nederl. Tijdschr. geneesk. 94:3541, 1950

26. Krahl, M. E: Hormones and the metabolism of isolated tissues, Diabetes 2:26, 1953.
27. Krahl, M E, and Park, C. R: The uptake of glucose by the isolated diaphragm of normal and hypophysectomized rats, J Biol Chem. 174 939, 1948.
28. Leupin, E, and Verzár, F.. Influence of desoxycorticosterone on glycogen formation and glucose uptake of isolated muscle, Nature, London 163.836, 1949.
29. Li, C H, Kalman, C, and Evans, H. M.: The effect of adrenocorticotropic and growth hormones on the glucose uptake and glycogen synthesis by the isolated diaphragm with and without insulin, Arch. Biochem 23 512, 1949
30. Li, C. H; Kalman, C., and Evans, H M.: The effect of hypophysectomy, adrenocorticotropic hormone, and adrenal cortical extract on the glucose uptake and glycogen synthesis by the isolated diaphragm with and without insulin, Arch Biochem. 22 357, 1949
31. Mirsky, A, and Broh-Kahn, R. H.. The inactivation of insulin by tissue extracts I. The distribution and properties of insulin inactivating extracts (insulinase), Arch. Biochem 20 1, 1949
32. Nelson, D H, and Samuels, L. T. 17-Hydroxycorticoids in peripheral blood of human subjects, J Clin Endocrinol. 11·1021, 1951.
33. Nelson, D H, and Samuels, L T A method for the determination of 17-hydroxycorticosteroids in blood 17-Hydroxycorticosterone in the peripheral circulation, J. Clin Endocrinol 12 519, 1952
34. Park, C. R, *et al.* The effect of growth hormone on glucose uptake by the isolated rat diaphragm, J Biol Chem. 197 151, 1952.
35. Perlmutter, M, and Greep, R. O Effect of insulin upon the in vitro glucose utilization and glycogenesis of the diaphragm of normal and pituitarectomized rats Adaptation of this technique as an assay for serum content of insulin and anti-insulin substances, Federation Proc 7·92, 1948
36. Perlmutter, M., and Greep, R O. The uptake of glucose and the synthesis of glycogen by the isolated diaphragm of normal and pituitectomized rats, J Biol Chem 174 915, 1948
37. Perlmutter, M, Weisenfeld S, and Mufson, M. "Bioassay of insulin in serum using the rat diaphragm" Endocrinology 50 442, 1952
38. Spoont, S, and Dyer, W W Insulin resistance associated with local and general allergy to insulin, J A M A 145 558, 1951
39. Stadie, W. C., and Zapp, J A, Jr The effect of insulin upon the synthesis of glycogen by rat diaphragm in vitro, J Biol Chem 170 55, 1947
40. Stadie, W C., Haugaard, N, and Marsh, J B Combination of epinephrine and 2,4-dinitrophenol with muscle of normal rat, J. Biol Chem 188 173, 1951.
41. Stadie, W. C, *et al*. Hormonal influences on the chemical combination of insulin with rat muscle (diaphragm), Am J M Sc 218 275, 1949
42. Takane, R: Über Atmung und Kohlehydratumsatz tierischer Gewebe, Biochem. Ztschr 171:403, 1926
43. Tuerkischer, E, and Wertheimer, E The in vitro synthesis of glycogen in

the diaphragms of normal and alloxan-diabetic rats, Biochem J. 42 603, 1948

44 Verzár, F, and Wenner, V.· The action of steroids on glycogen breakdown in surviving muscle, Biochem J 42.48, 1948

45 Verzár, F., and Wenner, V.. The influence in vitro of deoxycorticosterone on glycogen formation in muscle, Biochem J 42.35, 1948.

46 Villee, C A, and Hastings, A B. The metabolism of C^{14}-labeled glucose by the rat diaphragm in vitro, J Biol Chem 179 673, 1949

47. Waters, E. T., and Best, C H · The importance of developing an accurate method for the estimation of insulin in the blood, Proc Am Diab A 7 169, 1947.

48 Willebrands, A F., *et al.* Aanwijzingen voor de aanwezigheid van insuline in bloedserum, verkregen met behulp van het geisoleerde rattendiaphragma, Chem Weekblad 47.888, 1951.

49 Willebrands, A F, *et al*. Quantitative aspects of the action of insulin on the glucose and potassium metabolism of the isolated rat diaphragm, Science 112 277, 1950.

50 Willebrands, A F, *et al*: Unpublished data.

51 Yenerman, M, *et al*. Assay of insulin in blood, Federation Proc 12 162, 1953

Author Index

[Page numbers printed in italic indicate original contributions to this volume.]

T

U

V

W

Subject Index

A

Y

Z

Subject Index

A

D

Q

R

S

U

V

W

X

Z